# OPERATIONAL ENHANCEMENT OF AN UPFLOW ANAEROBIC SLUDGE BLANKET REACTOR TREATING FOG-REDUCED GRAIN DISTILLERY WASTEWATER

**HENDRIK SCHALK VAN DER WESTHUIZEN**

Thesis presented in partial fulfilment of the requirements for the degree of Master of Food Science in the Faculty of AgriSciences at Stellenbosch University


**Supervisor:** Dr G.O. Sigge

**Co-Supervisor:** Prof. T.J. Britz

April 2014

**DECLARATION**

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: 16 February 2014

## ABSTRACT

Waste generated by the distillery industry is a major ecological concern and disposal thereof without a suitable treatment can have damaging effects on the environment. The characteristics of this type of wastewater are highly variable and dependent on the raw material used and production process followed. Grain distillery wastewater (GDWW) is also rich in fats, oils and grease (FOG). Successful treatments of distillery wastewater and GDWW have been reported using an upflow anaerobic sludge blanket (UASB) reactor technology. The aim of this study was to investigate the ability of lab-scale UASB reactor to treat FOG-reduced GDWW and the subsequent enhancement thereof following an unique feeding strategy approach. Firstly, a coagulation/flocculation-centrifugation step was developed to obtain FOG-reduced GDWW. Secondly, the efficiency of a lab-scale UASB reactor was investigated treating FOG-reduced GDWW at pre-determined operational parameters as well as the verification of biomass acclimatisation. Lastly, the effect of a unique feeding strategy of FOG-reduced GDWW to lab-scale UASB reactor granules was investigated in terms of COD, FOG-reduction and biomass acclimatisation.

It was found that a coagulation/flocculation-centrifugation treatment removed sufficient amounts of FOG and TSS from GDWW. Different commercially available coagulation/flocculation products were evaluated whilst used in combination with a centrifugation step for improved sedimentation and separation. The FOG removal remained between 90 and 97% for the ferric chloride ($FeCl_3$) and Ferrifloc 1820 treatments, respectively, whereas the TSS removal ranged between 56 and 93%, respectively. The use of a high molecular weight polymer (Ultrafloc 5000) and an aluminium chlorohydrate (Ultrafloc 3800) proved to be less effective in terms of FOG removal efficiency, ranging from 72 to 86%. It was decided to pre-treat GDWW with $FeCl_3$ in combination with centrifugation to obtain FOG-reduced GDWW for subsequent UASB reactor treatment investigations.

The FOG-reduced GDWW was fed into a laboratory-scale UASB reactor (2 L) over a period of 331 days. During the operational period different feeding parameters were attained to establish the ability of the UASB reactor to efficiently treat FOG-reduced GDWW. The COD removal increased from 60 to 85% at an organic loading rate (OLR) of *ca.* 5.5 $kgCOD.m^{-3}.d^{-1}$ (pH = 7.5) whilst FOG removal remained between 45 and 70%. COD removal increased to 90% with the attainment of an OLR of *ca.*10 $kgCOD.m^{-3}.d^{-1}$ (pH = 7.5) whereas FOG removal remained in the region of 55 and 65%. COD and FOG removal remained above 85% and 50%, respectively, when substrate pH was decreased

to 6.50 (OLR *ca.* 10 kgCOD.$m^{-3}$.$d^{-1}$). A granule activity test was performed on seed and FOG-reduced GDWW fed granules to determine biomass acclimatisation. FOG-reduced GDWW fed granules showed higher activity in terms of methane production rate and cumulative methane production suggesting biomass acclimatisation.

The FOG-reduced GDWW was fed to a laboratory-scale UASB reactor following a unique feeding approach. The feeding approach consisted of several feeding and starvation cycles. Improved average biogas production was observed during the feeding (0.26 to 11.3 L.$d^{-1}$) and starvation (1.8 to 4.2 L.$d^{-1}$) cycles as higher loading rates were obtained during each feeding cycle. After the completion of the strategic feeding the UASB reactor was continuously fed at an organic loading rate of *ca.* 5 kgCOD.$m^{-3}$.$d^{-1}$. The COD reduction efficiency improved from 70 to 80%, however, FOG removal remained in the region of 60%. Granule activity tests done on days 0, 215 and 279 showed improved UASB granule activity to FOG-reduced GDWW with operation time in terms of methane production rate and cumulative methane production.

This study has proven that a coagulation/flocculation-centrifugation treatment of GDWW can remove sufficient amounts of FOG and TSS before the commencement of a UASB treatment, however, such a technique would require more refinement. It was also found that a UASB reactor can successfully treat FOG-reduced GDWW, however, it must be advised that close monitoring of the UASB reactor is required in order to maintain efficient COD reduction. A strategic feeding approach proved to be successful, but further improvement of the UASB efficiency to treat FOG-reduced GDWW in terms of stable COD and FOG reduction, stable effluent pH, improved biogas production and biomass activity must still be explored.

## OPSOMMING

Afloop water wat gegenereer word deur die distillerings-industrie veroorsaak ‘n ekologiese kommer en wegdoening daarvan sonder geskikte behandeling, kan ernstige gevolge op die omgewing hê. Die eienskappe van hierdie tipe afvalwater kan varieer en is afhanklik van die rou materiale gebruik en die produksie proses wat gevolg is. Graan distillery afloop water (GDAW) deel dieselfde eienskappe met die van distillery afloop water, alhoewel dit ook hoog is in vette, olies en ghries (VOG). Suksesvolle behandeling van distillery afloop water en GDAW met n opvloei-anaërobiese slykkombers (OAS) reaktor is deur verskeie navorsers gerapporteer. Die doel van hierdie studie was om die uitvoerbaarheid van laboratorium skaal OAS reaktor, wat VOG-verminderde GDAW behandel te ondersoek, asook die daaropvolgende verbetering deur n unieke voer strategie te volg. Eerstens, was ‘n koagulasie/flokkulasie-sentrifigasie tegniek ontwikkel om VOG-verminderde GDAW te kry. Tweendens, die effektiwiteit van ‘n lab-skaal OAS reaktor ondersoek, wat gevoer was met VOG-verminderde GDAW, by voorafbepaalde parameters. Laastens, die effek van ‘n unieke voer strategie van VOG-verminderde GDAW op lab-skaal OAS reaktor granules.

Dit was vasgestel dat ‘n koagulasie/flokkulasie-sentrifigasie voor behandeling voldoende hoeveelhede VOG en TSS verwyder van GDAW. Verskillende kommersieel beskikbare koagulasie/flokkulasie produkte was in kombinasie met ‘n sentrifugasie stap geëvalueer om sedimentasie en skeiding te verbeter. Dit was nie ‘n plan om die stap te perfek nie, maar dat dit eerder sou dien as ‘n voorbehandeling stap vir opeenvolgende ondersoeke. Die VOG verwydering het tussen 90 en 97% gevariëer vir ferri chloride ($FeCl_3$) en Ferrifloc 1820 (Chlorchem) en TSS verwydering het tussen 56 en 93% gewissel. Die gebruik van ‘n hoë molekulêre gewig polimeer (Ultrafloc 5000) en ‘n aluminium chlorohidraat (Ultrafloc 3800) was minder effektief met n VOG verwydering wat tussen 72 en 86% gewissel het.

Die VOG-verminderde GDAW was in ‘n laboratorium-skaal OAS reaktor oor ‘n tydperk van 331 dae behandel. Verskillende voer doelwitte was geëvaluaeer om te bepaal of ‘n OAS reaktor GDAW suksesvol kan behandel. CSB afbraak het van 60 to 85% gestyg teen ‘n organiese lading van 5.5 $kgCOD.m^{-3}.d^{-1}$ (pH 7.50), met VOG verwydering wat tussen 45 en 70% gewissel het. Die CSB afbraak het na die bereiking van 10 $kgCOD.m^{-3}.d^{-1}$ (pH 7.50) gestyg na 90% met VOG afbraak tussen 55 en 60% gewissel het. Die CSB en VOG verwydering het bo 85% en 50% onderskeidelik gebly, met die verlaging van substraat pH na 6.50 (CSB *ca.* 10 $kgCOD.m^{-3}.d^{-1}$). ‘n Aktiwiteits toets is uitgevoer met saad granules en VOG-verminderde GDAW gevoerde granules. Granules

(VOG-verminderde GDAW gevoer) het ‘n hoer aktiwiteit getoon teenoor saad granules in terme van metaan produksie tempo en kumulatiewe metaan produksie.

Die VOG-verminderde GDAW was gevoer in ‘n OAS reaktor deur gebruik te maak van ‘n strategiese voertegniek. Die strategie het uit verskeie voer en hongersnood fases bestaan. Verbeterde biogas produksie was tydens voer (0.26 tot 11.3 $L.d^{-1}$) en hongersnood (1.8 tot 4.2 $L.d^{-1}$) -fases opgelet soos ‘n hoër lading bereik was. Na die voltooing van die strategiese voer fase was die OAS reaktor op ‘n deurlopende basis teen ‘n lading van 5 $kgCOD.m^{-3}.d^{-1}$ gevoer. Die CSB verwydering het van 70 na 80% verhoog terwyl VOG afbraak in die omgewing van 60% gewissel het. Biomassa aktiwiteits toetse was uitgevoer is op dag 0, 215 en 279 het verhoogde aktiwiteit vertoon, met ‘n strategiese fase en deurlopende fase teenoor die aanvanklike (ongeaklamatiseerde) granules.

Hierdie studie het bewys dat ‘n flokkulasie/koagulasie-sentrifugasie behandeling van GDAW kan dien as ‘n voorbehandelings stap vir opeenvolgende OAS reaktor studies. Dit was gevind dat ‘n OAS reaktor die VOG-verminderde GDAW kan behandel, maar dit word aanbeveel dat die OAS reaktor so sorgvuldig as moontlik gemonitor word om effektiewe CSB verwydering te handhaaf. Ten slotte, ‘n strategiese voer strategie was suksesvol, maar verdere verbetering van die OAS reaktor ten opsigte van die behandeling van VOG-verminderde GDAW moet verder ondersoek word.

## ACKNOWLEDGMENTS

I would like to express my sincere gratitude to the following people and organisations for their invaluable contributions to the successful completion of this study:

Dr G.O. Sigge, as supervisor, for his expert guidance, encouragement, patience and support throughout this study.

Prof. T.J. Britz, as co-supervisor, for his enthusiasm, interest, expert advice and support throughout this study.

Distell and Stellenbosch University for providing the financial support to make this study an success.

Brink Liebenberg, Mare-Lou Prinsloo, Jacques Blignaut and Marlene Bester for providing the required wastewater, upflow anaerobic sludge blanket (UASB) granules and their keen interest throughout the study.

NCP Chlorchem® for providing the coagulation/flocculation products for this study.

Mrs. Daleen du Preez for their help on administrative duties.

Ashley Alfred Hendricks, fellow post-graduate student, for their invaluable and much appreciated assistance, as well as their friendship throughout this study.

Petro Mare du Buisson and Natasja Brown for their technical assistance.

Fellow post graduate students, friends, family for their support, interest, motivation and understanding throughout this study.

Finally, God the Almighty, who made it all possible and gave me the strength to see it through.

# CONTENTS

The language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This dissertation represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF ACRONYMS

| | |
|---|---|
| ATM | Acetic Test Media |
| BOD | Biochemical Oxygen Demand |
| BTM | Basic Test Media |
| CSB | Chemiese Suurstof Behoefte |
| COD | Chemical Oxygen Demand |
| FOG | Fats, Oils and Grease |
| GDAW | Graan Distillery Afloop Water |
| GDWW | Grain Distillery Wastewater |
| GTM | Glucose Test Media |
| HRT | Hydraulic Retention Time |
| LCFA | Long Chain Fatty Acids |
| OLR | Organic Loading Rate |
| OAS | Opvloei-anaërobiese Slykkombers |
| TSS | Total Soluble Solids |
| TSS | Totale Suspendeerde Soliedes |
| UASB | Upflow Anaerobic Sludge blanket |
| VFA | Volatile Fatty Acids |
| VOG | Vette, Olies en Ghries |

# CHAPTER 1

## INTRODUCTION

The continuous expansion of various industrial and urban sectors has created a lot of pressure on the sustainability of water systems. The geographic distribution of water resources does not always correspond to the location of the demand centres, thus making the management of water critical (Perret, 2002; Otieno & Ochieng, 2007; Adewumi *et al.*, 2010). Waste generated by industries is a major ecological concern and disposal of effluent without the suitable treatment could have damaging effects on the environment (Adewumi *et al.*, 2010). Thus, the deteriorating water supplies and quality are major threats to South Africa's capability to provide sufficient water to meet its demands and to ensure environmental sustainability (Otieno & Ochieng, 2007; Adewumi *et al.*, 2010). It is essential to understand that South Africa's water supply is limited and the use of it must proceed as efficiently as possible.

Governments worldwide, including South Africa, are setting more strict requirements for pollution control creating a demand for more effective and novel treatment technologies (Lu *et al.*, 1995; Akunna & Clark, 2000; Mohana *et al.*, 2009). Responsible management of effluents requires that their potential environmental impacts be minimal in addition to being within an acceptable range, and with new understandings and developments, the treatment objectives have shifted (Gogate, 2002; Kirzhner *et al.*, 2008). Treatments should be eco-friendly, flexible enough to handle changes in the loading rates, have low initial capital costs and be easily operated and maintained throughout without impacting removal efficiency (Kirzhner *et al.*, 2008).

The distillery industry can be classified as a high polluting industry due to the volume and strength of the stillage (wastewater) produced annually (Nataraj *et al.*, 2006; Sowmeyan & Swaminathan, 2008; Mohana *et al.*, 2009). Water is a key process medium in this industry and is used for preparation, cleaning, sanitation, heating, cooling, floor washing, etc. (Willey, 2001; Nataraj *et al.*, 2006; Sarkar *et al.*, 2006). Distilleries are one of the highest consumers of raw water with consumption ranging from 25 to 175 L for every litre of alcohol produced (Nataraj *et al.*, 2006). Furthermore the amount of wastewater produced is nearly 15 times that of the total alcohol production (Sowmeyan & Swaminathan, 2008). If this wastewater is left untreated it can have severe environmental implications (Satyawali & Balakrishnan, 2007; Mohana *et al.*, 2009). The production and characteristics of this type of wastewater are highly variable and dependent on the raw

material used and the type of ethanol production process (Mohana *et al.*, 2009). The wastewater is characterised by having a high concentration of chemical oxygen demand (COD) and biochemical oxygen demand (BOD), low pH, foul odour and a dark brown colour (Satyawali & Balakrishnan, 2007; Sowmeyan & Swaminathan, 2008; Mohana *et al.*, 2009). Furthermore, the inorganic compounds (nitrogen, potassium, phosphates, calcium and sulphates) in the spent-wash are also very high (Mohana *et al.*, 2009). Grain distillery wastewater shares the same characteristics to that of distillery wastewater with COD ranging from 10 000 to 60 000 $mg.L^{-1}$ (Goodwin & Stuart, 1994; Gao *et al.*, 2007). However, it is also rich in fats, oils and grease (FOG), ranging from 1 000 to 2 000 $mg.L^{-1}$ (Gie, 2007).

The upflow anaerobic sludge blanket (UASB) reactor has become a popular efficient and versatile anaerobic treatment system operated throughout the world. The system presents an attractive solution because of a low operational cost, low energy consumption, compact design, low sludge production and production of methane ($CH_4$) as a potential energy source (Lettinga *et al.*, 1980; Forday & Greenfield, 1983; Goodwin *et al.*, 1990; Chernicharo, 2007). The UASB reactor operates as a suspended growth system (without the use of any packing material) with the active biomass in the form of granules held in suspension by hydraulic design (Deepak, 1998; Tiwari *et al.*, 2006).

Successful treatment of a wide variety of different wastes including those from the sugar industry, distillery and brewery has led to more than a 1 000 UASB units being utilized by different industries all over the world (Droste, 1997; Gavrilescu, 2002; Chernicharo, 2007). These systems can be used as a single treatment step or in combination with a pre-treatment or post-treatment step. Goodwin (1994) was able to successfully treat grain distillery wastewater (GDWW) at a loading rate of 15 $kgCOD.m^{-3}.d^{-1}$. Uzal *et al.* (2003) used a two-stage UASB system to reduce up to 93% of the COD from distillery wastewater and further increased the COD reduction up to 99% during a subsequent aerobic treatment. Gao *et al.* (2007) successfully treated GDWW and achieved up to 97.3% COD reduction at an OLR between 5 and 48 $kgCOD.m^{-3}.d^{-1}$ with a HRT of 82 to 11 h. Gie (2007) was able to successfully treat wine distillery wastewater by combining a pre-ozonation step with a subsequent UASB treatment. The substrate COD, at a loading rate of 4 000 $mg.L^{-1}$, was reduced to *ca.* 320 $mg.L^{-1}$ (92%) effluent COD (Gie, 2007).

The high lipid content of GDWW is, however, often associated with problems during biological treatment, especially anaerobic treatment (Cavaleiro *et al.*, 2007). These operational problems are a result of the accumulation of lipids onto the microbial

aggregates by mechanisms of adsorption, precipitation and entrapment (Cavaleiro *et al.*, 2007). The adsorption of lipids onto the biomass can alter the sludge's ability to settle and can lead to sludge bed washout. Accumulation can also create a physical barrier that hinders the transfer of substrates and metabolic products (Cavaleiro *et al.*, 2001; Cavaleiro *et al.*, 2007; Chipasa & Mdrzycka, 2008). Long chain fatty acids (LCFA), intermediates of lipid metabolism, have been reported to have inhibitory effects on acetoclastic methanogens and acetogens (Koster & Cramer, 1987; Rinzema *et al.*, 1994; Mendes & Castro, 2005; Miranda *et al.*, 2005). This may severely hinder the effectiveness of an UASB reactor to treat FOG-rich GDWW and an efficient pre-treatment is required in order to reduce the excess FOG in this type of wastewater.

A coagulation/flocculation treatment is one of the most significant physico-chemical steps to remove soluble solids and colloidal material contributing to turbidity, COD and BOD of wastewater (Al-Mutairi *et al.*, 2004; Sarkar *et al.*, 2006). Coagulation/flocculation is normally employed to treat wastewater containing high amounts of small particles (< 5 µm) and fats and involves combining these particles (colloidal or suspended) and other organic material into larger aggregates, thereby facilitating sedimentation or flotation (Hogg, 2000; Zhou *et al.*, 2008). The effectiveness of this treatment will depend on the coagulation/flocculation agent used, dosage strength, pH and ionic strength of the solution and the concentration and nature of the organic compounds in the wastewater (Dominguez *et al.*, 2005; Zayas *et al.*, 2007). Zayas *et al.* (2007) showed that increased pH can improve the efficiency when treating vinasse with a combined coagulation/flocculation-electrochemical oxidation treatment. The COD removal increased from 54% (pH 4.0 - 6.0) to 84% (pH 6.0 - 8.4) using $FeCl_3$ (20 $g.L^{-1}$) as coagulant (Zayas *et al.*, 2007).

The objective of this study is to enhance the efficiency of an UASB reactor treating FOG-reduced GDWW. This will be done by firstly using a coagulation/flocculation-centrifugation step to obtain FOG-reduced GDWW. Secondly, to optimise the efficiency of a lab-scale UASB reactor treating the FOG-reduced GDWW at pre-determined operational parameters (increased OLR and lower influent pH). At the same time the level of biomass acclimatisation, in terms of granule activity, will also be monitored. Thirdly, the stability of the granules in the UASB will be optimised by investigating the effect of a strategic feeding approach on the COD and FOG degradation in the lab-scale UASB reactor.

## REFERENCES

Adewumi, J.R., Ileombade, A.A. & Zyl, J.E.v. (2010). Treated wastewater reuse in South Africa: Overview, potential and challenges. *Resources, Conservation and Recycling*, **55**, 221-231.

Akunna, J.C. & Clark, M. (2000). Performance of a granular-bed anaerobic baffled reactor (GRABBR) treating whisky distillery wastewater. *Bioresource Technology*, **74**, 257-261.

Al-Mutairi, N.Z., Hamoda, M.F. & Al-Ghusain, I. (2004). Coagulant selection and sludge conditioning in a slaughterhouse wastewater treatment plant. *Bioresource Technology*, **95**, 115-119.

Cavaleiro, A., Alves, J. & Alves, M. (2007). Discontinuous operation promotes efficient continuous anaerobic treatment of effluents with high lipid content. In: 11th IWA World Congress on Anaerobic Digestion. Brisbane, Australia.

Cavaleiro, A.J., Alves, M.M. & Mota, M. (2001). Microbial and operational response of an anaerobic fixed bed digester to oleic acid overloads. *Process Biochemistry*, **37**, 387-394.

Chernicharo, C.d.L. (2007). *Anaerobic Reactors.* Pp. 1 - 184. IWA Publishing.

Chipasa, K.B. & Mdrzycka, K. (2008). Characterization of the fate of lipids in activated sludge. *Journal of Environmental Sciences*, **20**, 536-542.

Deepak, L.J. (1998). High rate anaerobic treatment of industrial wastewater in tropics. *International Journal of Science and Technology*, **3**, 1 - 7.

Dominguez, J., de Heredia, J., Gonzalez, T. & Sanchez-Lavado, F. (2005). Evaluation of ferric chloride as a coagulant for cork processing wastewaters. Influence of the operating conditions on the removal of organic matter and settleability parameters. *Industrial and Engineering Chemistry Research*, **44**, 6539-6548.

Droste, R. (1997). *Theory and practice of water and wastewater treatment.* Wiley Press.

Forday, W. & Greenfield, P.F. (1983). Anaerobic digestion. *Effluent and Water Treatment Journal*, **23**, 405-409.

Gao, M., She, Z. & Jin, C. (2007). Performance evaluation of a mesophilic (37°C) upflow anaerobic sludge blanket reactor in treating distiller's grains wastewater. *Journal of Hazardous Materials*, **141**, 808-813.

Gavrilescu, M. (2002). Engineering concerns and new developments in anaerobic waste-water treatment. *Dynamic Biochemistry, Process Biotechnology and Molecular Biology*, **3**, 346-362.

Gie, L.M. (2007). *Effect of pre-treated distillery wastewater on upflow anaerobic sludge blanket granules and reactor efficiency*. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Gogate, P.R. (2002). Cavitation: an auxiliary technique in wastewater treatment schemes. *Advances in Environmental Research*, **6**, 335-358.

Goodwin, J.S. & Stuart, J.B. (1994). Anaerobic digestion of malt whisky distillery pot ale using upflow anaerobic sludge blanket reactors. *Bioresource Technology*, **49**, 75-81.

Goodwin, J.S., Wase, D.J. & Forster, C.F. (1990). Effects of nutrient limitation on the anaerobic upflow sludge blanket reactor. *Enzyme and Microbial Technology*, **12**, 877-884.

Hogg, R. (2000). Flocculation and dewatering. *International Journal of Mineral Processing*, **58**, 223-236.

Kirzhner, F., Zimmels, Y. & Shraiber, Y. (2008). Combined treatment of highly contaminated winery wastewater. *Separation and Purification Technology*, **63**, 38-44.

Koster, I. & Cramer, A. (1987). Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids. *Applied & Environmental Microbiology*, **53**, 403-409.

Lettinga, G., van Velsen, A.F.M., Hobma, S.W., de Zeeuw, W. & Klapwijk, A. (1980). Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnology and Bioengineering*, **22**, 699-734.

Lu, C., Yeh, A.C. & Lin, M.-R. (1995). Treatment of high-strength organic wastewaters using an anaerobic rotating biological contactor. *Environment International*, **21**, 313-323.

Mendes, A. & Castro, H. (2005). Effect on the enzymatic hydrolysis of lipids from dairy wastewater by replacing Gum Arabic emulsifier for sodium chloride. *Brazilian Archives of Biology and Technology*, **48**, 135-142.

Miranda, L., Henriques, J. & Monteggia, L. (2005). A full-scale UASB reactor for treatment of pig and cattle slaughterhouse wastewater with a high oil and grease content. *Brazilian Journal of Chemical Engineering*, **22**, 601-610.

Mohana, S., Acharya, B.K. & Madamwar, D. (2009). Distillery spent wash: Treatment technologies and potential applications. *Journal of Hazardous Materials*, **163**, 12-25.

Nataraj, S.K., Hosamani, K.M. & Aminabhavi, T.M. (2006). Distillery wastewater treatment by the membrane-based nanofiltration and reverse osmosis processes. *Water Research*, **40**, 2349-2356.

Otieno, F. & Ochieng, G. (2007). Water management tools as a means of averting a possible water scarcity in South Africa by the year 2025. *Water SA*, **30**, 120-124.

Perret, S. (2002). Water policies and smallholding irrigation schemes in South Africa: a history and new institutional challenges. *Water Policy*, **4**, 283-300.

Rinzema, A., Boone, M., van Knippenberg, K. & Lettinga, G. (1994). Bactericidal effect of long chain fatty acids in anaerobic digestion. *Water Environment Research*, **66**, 40-49.

Sarkar, B., Chakrabarti, P., Vijaykumar, A. & Kale, V. (2006). Wastewater treatment in dairy industries-possibility of reuse. *Desalination*, **195**, 141-152.

Satyawali, Y. & Balakrishnan, M. (2007). Removal of color from biomethanated distillery spentwash by treatment with activated carbons. *Bioresource Technology*, **98**, 2629-2635.

Sowmeyan, R. & Swaminathan, G. (2008). Effluent treatment process in molasses-based distillery industries: A review. *Journal of Hazardous Materials*, **152**, 453-462.

Tiwari, M., Guha, S., Harendranath, C. & Tripathi, S. (2006). Influence of extrinsic factors on granulation in UASB reactor. *Applied Microbiology and Biotechnology*, **71**, 145-154.

Willey, R. (2001). Fats, oils, and greases: The minimization and treatment of wastewaters generated from oil refining and margarine production. *Ecotoxicology and Environmental Safety*, **50**, 127-133.

Zayas, T., Rómero, V., Salgado, L., Meraz, M. & Morales, U. (2007). Applicability of coagulation/flocculation and electrochemical processes to the purification of biologically treated vinasse effluent. *Separation and Purification Technology*, **57**, 270-276.

Zhou, Y., Liang, Z. & Wang, Y. (2008). Decolorization and COD removal of secondary yeast wastewater effluents by coagulation using aluminum sulfate. *Desalination*, **225**, 301-311.

# CHAPTER 2

# LITERATURE REVIEW

## A. WATER MANAGEMENT IN SOUTH AFRICA

### INCREASING WATER SCARCITY

Sustainable water development and management is critical for the development of all societies, however, the geographic distribution of water resources does not always correspond to the location of the demand centres (Otieno & Ochieng, 2007; Adewumi *et al.*, 2010). South Africa may be defined as a water scarce country due to its low average annual precipitation of less than 500 mm (Perret, 2002; Otieno & Ochieng, 2007; Adewumi *et al.*, 2010). This value is well below the world average rainfall of 860 mm per annum (Otieno & Ochieng, 2004; Otieno & Ochieng, 2007). South Africa is forecasted to experience water scarcity by the year 2025 with annual freshwater availability of less than 1000 $m^3$ per capita (Otieno & Ochieng, 2007). South Africa's water resources are in global terms scarce and limited in extent. The unique climate and geography of South Africa is strongly influenced by seasonal rainfall and uneven availability of water, 21% of the country receiving less than 200 mm, results in only 8.6% of all rainfall being converted to usable runoffs (Perret, 2002; Otieno & Ochieng, 2007; Adewumi *et al.*, 2010). The continuous developing and expanding industrial and urban sectors together with more than 1.3 million hectares used for agricultural purposes has put a lot of pressure on the sustainability of water systems in South Africa (Perret, 2002; Otieno & Ochieng, 2007) (Otieno & Ochieng, 2007; Adewumi *et al.*, 2010). Deteriorating water quality is one of the major threats to South Africa's ability to provide sufficient water (of appropriate quality) to meet its needs as well as to ensure environmental sustainability (Otieno & Ochieng, 2007). It is thus essential to understand that South Africa's water supply is limited and the use of it must proceed as efficiently and cleanly as possible.

### INCREASED PREASURE CREATED BY INDUSTRIALISATION

Industrialisation of land can be considered as a desirable option owing to its economical contribution, though it exerts considerable pressure on natural resources along with

increased demand in energy (Lata *et al.*, 2002; Kirzhner *et al.*, 2008). Waste generated by industries is a major ecological concern and disposal of effluent without the suitable treatment could have long term adverse effects especially on the local vegetation and aquatic life (Lata *et al.*, 2002). Water availability is becoming a challenging problem in societies in some regions all over the world and the rapid population growth together with increasing water withdrawal for agricultural use culminates in a large population suffering from water deficits (Rivas *et al.*, 2009). Governments worldwide, including South Africa, are setting more strict requirements for pollution control and there has been an increasing demand for more effective and novel treatment technologies (Lu *et al.*, 1995; Akunna & Clark, 2000; Mohana *et al.*, 2009). In the past the objective of wastewater treatment was concerned with the removal of soluble solids, floatable materials and the removal of pathogens. Responsible management of effluents requires that their potential environmental impacts be minimal in addition to being within an acceptable range and with new understandings and developments, the treatment objectives have shifted (Gogate, 2002; Kirzhner *et al.*, 2008). Several criteria have to be studied before deciding on a treatment: The process should be eco-friendly, flexible to handle changes in the loading rates, have low initial capital investing and be easily operated and maintained (Kirzhner *et al.*, 2008). It is thus essential for highly polluting industries to adopt a suitable waste treatment process for the clean disposal of high strength wastewater.

## B. HIGH POLLUTING INDUSTRIES

Due to the increasing scarcity of clean water, there exists a demand for the reuse of the treated wastewater, residues deriving thereof and other by-products (Aiyuk *et al.*, 2006). Due to the development of various industrial sectors, for example the beverage, textile, electronics and food, large volumes of wastewaters are produced during these processes (Kuang, 2002; Piya-Areetham *et al.*, 2006). Water is a key process medium to most of the industries and is used for preparation, processing, cleaning, sanitation, heating, cooling, floor washing, etc. (Willey, 2001; Nataraj *et al.*, 2006; Sarkar *et al.*, 2006).

The distillery industry can be classified as a high-polluting industry due to the amount and strength of the stillage (wastewater) produced annually (Nataraj *et al.*, 2006; Mohana *et al.*, 2008; Sowmeyan & Swaminathan, 2008). Alcohol distilleries are rapidly expanding to meet the ever increasing demand worldwide (Mohana *et al.*, 2008). Distilleries are one of the highest consumers of fresh water, with consumption ranging from 25 to 175 L for every litre of alcohol produced (Nataraj *et al.*, 2006). Furthermore the

amount of wastewater produced is nearly 15 times that of the total alcohol production (Sowmeyan & Swaminathan, 2008). Characterised by its high strength, if this wastewater is left untreated it can result in severe environmental implications (Satyawali & Balakrishnan, 2007; Mohana *et al.*, 2009).

## THE SOUTH AFRICAN LIQUOR INDUSTRY

The South African liquor industry comprises of beer, wine and spirit segments. The entire liquor market is served by only a handful of competitors. The liquor industry makes a significant contribution to the South African economy such as the payment of company taxes, provider of employment, supplier and user of a variety of goods and services and a role player in the tourist industry. The liquor industry in South Africa was estimated at a revenue of R52 billion in 2002 with 18,4 million litres alone of whisky produced during 2002/2003 worth R1.6 billion and covering 13.3% of the liquor market share in South Africa (Clare *et al.*, 2004; Naumann, 2005; Kriel, 2010). This revenue is projected to grow by 5% annually (Kriel, 2010). In 2008 this liquor market share increased to 24.5%, totalling 3.3% of the total market share in South Africa (SAWIS, 2009). New entrants into the market, increased demand (locally and globally) and the development of new products have all radically increased the rate of production as well as water utilisation.

## DISTILLING INDUSTRY AND WHISKEY PRODUCTION PROCESS

Various substrates including sugar crops (sugar beets, sugar cane, molasses, etc.), starch crops (corn, wheat, rice, cassava, etc.), dairy (whey) and cellulosic materials may be used for alcohol production (Wilkie *et al.*, 2000; Mohana *et al.*, 2008; Satyawali & Balakrishnan, 2008). Whisky is produced all over the world and although the production is in essence the same, the product has taken on numerous guises depending on the country in which it is produced and the grain used for production (Anonymous, 2009; Csar, 2009). Irish and American whiskey differs from Scottish whisky only by the spelling (spelt with an ‘e’). Whisky is prepared from fermented cereals which are further matured in oak barrels. The cereals used for whisky production include corn, rye, barley, maize and wheat. The production process involves malting, mashing, fermentation, distillation and maturation (Goodwin & Stuart, 1994; APHA, 1998; Goodwin *et al.*, 2001; Uzal *et al.*, 2003; Csar, 2009). Traditionally maize was the grain of choice for Scottish whisky until it was replaced by wheat during the 1980’s in Scotland due to its economic value (Agu *et al.*, 2006).

However, maize is still considered to be superior over wheat as it produces higher alcohol yields and presents fewer processing problems (Agu *et al.*, 2006; Agu *et al.*, 2008). In South Africa maize is the most important crop and is produced throughout the country in diverse environments. Approximately 8 million tons is produced in South Africa annually on approximately 3.1 million Ha of land (du Plessis, 2003).

Malting involves the steeping of the cereal in the water until the onset of germination. This releases the enzymes responsible for the breakdown of starches to fermentable sugars. The objective of mashing is to render and liquefy as much of the valued content of the malt as possible. Water at different temperatures is used to achieve a sugary liquid known as wort. Yeast is added to the wort to allow fermentation for 48 hours. Distillation in a Coffey still of the wash (fermented wort) increases the alcohol content and removes impurities. The final product after distillation is matured in oak caskets. All of these steps contribute differently to the final strength of wastewater produced (Goodwin & Stuart, 1994; APHA, 1998; Goodwin *et al.*, 2001; Uzal *et al.*, 2003; Csar, 2009).

## DISTILLERY WASTEWATER

Large amounts of the stillage are produced annually during ethanol production from various materials with nearly 61% of the world's ethanol production from sugar cane (Mohana *et al.*, 2008). The production and characteristics of this type of wastewater are highly variable and dependent on the raw material used and the type of ethanol production process (Mohana *et al.*, 2008). The wastewater is characterised by having a high amount of organic material (high COD and BOD), high solids, low pH, foul odour and a dark brown colour (Satyawali & Balakrishnan, 2007; Sowmeyan & Swaminathan, 2008; Mohana *et al.*, 2009). Furthermore, the inorganic substances (nitrogen, potassium, phosphates, calcium and sulphates) in the spent wash are also very high (Mohana *et al.*, 2008). The brown colour of the wastewater is related to melanoidins. These polymers have antioxidant properties which may be toxic to the microorganisms typically used in biological wastewater treatment processes (Mohana *et al.*, 2008). Disposal of these types of wastewaters untreated or partially treated can be hazardous to the environment. Depletion of the oxygen related to the proliferation of the microbial population in natural water bodies can lead to the widespread mortality of fish and other aquatic organisms (Hati *et al.*, 2007). Disposal onto the soil can lead to acidification (Mohana *et al.*, 2008).

Spent wash and spent lees are the liquid residues after distillation has taken place during whisky production. These residues together with the water used during the production results in the effluent produced (Goodwin & Stuart, 1994). Also known as grain distillery wastewater (GDWW), this effluent can have detrimental effects on the environment if not treated sufficiently. Grain distillery wastewater shares the same characteristics of other distillery wastewaters, however, it is also rich in fats oils and grease (FOG). The different constituents of distillery wastewater and GDWW are summarised in Table 1.

**Table 1.1** The composition of distillery wastewater and GDWW (Goodwin & Stuart, 1994; Tokuda *et al.*, 1998; Uzal *et al.*, 2003; Gao *et al.*, 2007).

| Constituent | | Distillery wastewater | GDWW |
|---|---|---|---|
| COD | $mg.L^{-1}$ | 110 000 – 190 000 | 10 000 – 60 000 |
| $BOD_5$ | $mg.L^{-1}$ | 50 000 – 60 000 | 15 000 – 34 000 |
| Total solids | $g.L^{-1}$ | 110 – 190 | 20 – 52 |
| Total suspended solids | $g.L^{-1}$ | 13 – 15 | 10 – 11 |
| Total dissolved solids | $g.L^{-1}$ | 90 – 150 | - |
| Volatile suspended solids | $mg.L^{-1}$ | 80 – 120 | 160 – 640 |
| Total phosphorous | $mg.L^{-1}$ | - | 15.0 – 18.0 |
| Total Nitrogen | $mg.L^{-1}$ | 5 – 7 | 120 – 150 |
| Chloride | $(g.L^{-1}$ | 8.0 – 8.5 | - |
| Phenols | $g.L^{-1}$ | 8 – 10 | - |
| pH | - | 3.0 – 4.5 | 3.5 – 4.0 |

## C. POSSIBLE TREATMENT OPTIONS

### PHYSICAL TREATMENT OF WASTEWATER

*Sedimentation*

An inexpensive method used to treat wastewater where solids may be removed from the carrier fluid by gravitational forces (Jayanti & Narayanan, 2004). Although not effective at improving clarity or removing microorganisms, sedimentation can be used to control particulate pollutants and may serve as a pre-treatment or post treatment of wastewater (Hogg, 2000; White & Verdone, 2000). Continuous sedimentation systems are now

employed to meet the high throughput of wastewater production. A typical sedimentation tank consists of a large shallow circular tank with an inclined bottom, a rake mechanism is fitted to scrape the settled sludge while a outlet weir at the side of the tank facilitates overflow. The wastewater is either fed from the bottom or top (Jayanti & Narayanan, 2004). Free settling, hindered settling or compression are the mechanisms followed to achieve settling in sedimentation tanks (Hogg, 2000). Efficiency of sedimentation depends on the characteristics of the suspended solids (particle size, density, settling velocity and concentration of solids), flow field and geometrical dimensions of the tank (Fan *et al.*, 2007). Sedimentation may also be enhanced by a flocculation step (Hogg, 2000). Reduction in COD of up to 82% were achieved by Beltrán *et al.* (2001) when distillery wastewater was treated in a system consisting of an aeration tank, 4 L mixing tank, feed and effluent reservoirs and a sedimentation tank.

*Granular media filters*

Granular media filters present an economical solids-liquid separation practice to achieve preferred water quality standards with respect to the particulate parameters (Boller & Kavanaugh, 1995). A typical filter consists of sand, with the grains having a variety of shapes and none are spherical. These grains do not rest against one another in a structured manner so pores between the pellets can vary in size from closely packed triangles to larger cubical shapes. This wide variety of pores each with individual shapes and sizes form a unique three dimensional filter (Boller & Kavanaugh, 1995; Stevenson, 1997). Numerous studies have shown granular media filtration capable of treating a variety of wastewater types (Boller & Kavanaugh, 1995). The effectiveness of the filter depends on the physical parameters (size and shape of granular media, depth of media, clean bed porosity), physical characteristics of suspension (particle size, particle distribution, concentration, shape and density), surface chemistry of media and particulate (pH, ionic strength) and surface properties of media and particulate (Boller & Kavanaugh, 1995). Loading rate should also be considered and plays an important role in the effectiveness of granular media filtration (Williams *et al.*, 2007). Granular media filters can be used as a cheap polishing step during distillery wastewater treatment (Ripley, 1979).

*Membrane separation techniques*

Successful treatments of highly contaminated wastewater using membrane separation techniques have been achieved by different researchers (Wilkie *et al.*, 2000; Lapisova *et al.*, 2006; Melamane *et al.*, 2007). The most popular technologies in use include

nanofiltration, ultrafiltration and reverse osmosis (Nataraj *et al.*, 2006). These technologies are applied where stringent discharge standards are necessary. These systems are capable of effective treatments of various types of wastewaters in a standalone setup. This technology can also be used in hybrid with a biological process to enhance the efficiency while saving on capital costs (Nataraj *et al.*, 2006; Melamane *et al.*, 2007). Problems such as dangers of scaling, membrane compacting, increased energy consumption and operational costs may arise when using the technology by itself when trying to achieve 100% reduction efficiency (Rautenbach *et al.*, 2000; Wilkie *et al.*, 2000). Membrane-bioreactors may yield advantages such as better biomass retention, allowing higher organic loading rates, higher quality effluent, more compact design and the complete reduction of solids (Rautenbach *et al.*, 2000).

Different hybrid technologies were used by researchers treating a variety of wastewaters. Fuchs *et al.* (2003) used a cross flow membrane bioreactor when treating animal slaughterhouse effluent at 0.8 $kgCOD.L^{-1}.d^{-1}$ achieving COD reduction consistently above 90%. Using hybrid nanofiltration and reverse osmosis technologies Nataraj *et al.* (2006) was able to successfully remove colour and contaminants from distillery spent wash by up to 99.8% total dissolved solids (TDS) reduction and 99.9% COD reduction. Lapisova *et al.* (2006) treated distillery stillage using a 3 channel ceramic membrane (0.2 µm) supplemented by ultrafiltration (15 and 50 kDa) to achieve 80% COD reduction efficiency.

## PHYSICO – CHEMICAL TREATMENT OF WASTEWATER

*Ultrasound treatment*

Ultrasound has been well recorded for the treatment of a variety of different wastewater types (Gogate, 2002). Also known as sonochemical oxidation, ultrasound results in the phenomena known as acoustic cavitation (Gogate, 2002; Gonze *et al.*, 2003; Sangave & Pandit, 2004). Cavitation can be defined as a phenomenon of formation, growth and subsequent destruction of millions of micro bubbles or cavities over small time intervals (milliseconds). This results in the release of large amounts of energy in a very small location. The end result is the formation of oxidising species such as hydroxyl radicals ($OH^{-}$) and hydrogen peroxide ($H_2O_2$) together with a high temperature and pressure. The contaminants get completely or partially oxidised almost instantaneously (Gogate, 2002; Gonze *et al.*, 2003; Sangave & Pandit, 2004). Although an expensive treatment, ultrasound effectively degrades complex organic compounds enhancing biodegradation.

Sangave and Pandit (2004) found that ultrasound as only pre-treatment increased the aerobic oxidation of distillery spent wash from 25 to 44% COD reduction but concluded that a high time scale requirement for effective mineralisation was economically susceptible and recommended using ultrasound as part of a hybrid pre-treatment step. The use of ultrasound followed by enzymatic pre-treatment increased COD reduction of distillery spent wash from 34.9 to 62.5% during aerobic oxidation (Sangave & Pandit, 2006b)

*Coagulation/Flocculation*

The coagulation/flocculation treatment is one of the most important physico-chemical steps to reduce soluble solids and colloidal material which may contribute to wastewater turbidity as well the reduction of COD and BOD content of the wastewater (Al-Mutairi *et al.*, 2004; Sarkar *et al.*, 2006). Coagulation/flocculation is normally required to treat wastewater containing high amounts of small particles (< 5 μm) and it involves combining these particles (colloidal or suspended) and other organic material into larger aggregates, thereby facilitating the sedimentation or flotation of these flocs (Hogg, 2000; Zhou *et al.*, 2008). If economically viable the coagulation/flocculation agents can be recovered from the sludge produced during treatment and reused (Aguilar *et al.*, 2002).

There are various types of coagulation/flocculation agents and these may be classified into different groups namely inorganic (aluminium sulphate, aluminium chloride, polyaluminium sulphate, polyaluminium chloride, ferric chloride, ferric sulphate, ferrous sulphate), organic polymers, microbial (extracellular biopolymeric flocculants) and naturally occurring agents (chitosan, starches, tannins, alginates) (Salehizadeh & Shojaosadati, 2001; Dominguez *et al.*, 2005).

The process of coagulation involves the destabilisation of the anionically charged suspended colloidal materials (Chesters *et al.*, 2009). Destabilisation can be a result of charge neutralisation or the enmeshment in a metal hydroxide precipitate (Zhou *et al.*, 2008). Flocculation involves the aggregation of the particles into micro-flocs and subsequently larger flocs. There are two different mechanisms that will determine the rate of flocculation rate, i.e. perikinetic and orthokinetic-flocculation (Oppel, 1987). Perikinetic flocculation takes place spontaneously and occurs due to the Brownian diffusion or thermal agitation. This type of flocculation is not applicable to particles larger than 10 μm. Orthokinetic flocculation is a non-spontaneous process and will only arise when a mechanical energy (by means of mixing) is applied to the solution (Oppel, 1987). Formed flocs will either float or sink, making them easier to remove from the system (Chesters *et*

*al.*, 2009). Some coagulants have the ability to perform both coagulation and flocculation functions at once. Although the primary function of coagulation is to ensure charge neutralisation of the colloids it can often absorb onto several colloids resulting in bridge formation and subsequently resulting in the colloids to flocculate. The mechanism of coagulation/flocculation can be summarised as followed: Charge neutralisation, Bridging and Colloid entrapment. Charge neutralisation is the result of the adsorption of the positively charged coagulant ions onto the negatively charged surface of the colloid. This technique is carefully controlled by the coagulant dosage. Overdosing can reverse the charge and subsequently redisperse the colloid and result in poor flocculation (Bratby, 2006). Bridging is the result of the coagulant or flocculant forming fibres to attach several colloids, binding them together. High molecular weight coagulant/flocculants are more effective at bridging. In practice charge neutralisation with a low molecular weight coagulant will be followed by a polymeric flocculant to ensure the growth of fast and shear resistant flocs. Colloid entrapment occurs when excess amounts of coagulants (usually low molecular weight) are added to the solution, exceeding the required amount for charge neutralisation. The result is the formation of hydrous metal oxide precipitates which will entrap most of the colloids. This mechanism is also known as the "sweep mechanism" (Bratby, 2006).

The effectiveness of this treatment will depend on the coagulation/flocculation agent used, dosage, pH and ionic strength of the water and the concentration and nature of the organic compounds in the wastewater (Dominguez *et al.*, 2005; Zayas *et al.*, 2007). Particles within the solution carry a charge due to electrochemical interactions between the particles and surrounding solution and this charge is influenced by the solution's pH. Thus, pH control can greatly affect the coagulation efficiency of the flocculant (Hogg, 2000). The pH must stay in such a range to ensure solubility of the metal as well as the hydroxide in the solution. High pH values will not induce restabilisation regardless of the colloid concentration or coagulant dosage. Zayas *et al.* (2007) showed that increased pH can improve the efficiency when treating vinasse with a combined coagulation/flocculation-electrochemical oxidation treatment. The COD removal increased from 54% (pH 4 - 6) to 84% (pH 6 - 8.4) using $FeCl_3$ (20 $g.L^{-1}$) as coagulant (Zayas *et al.*, 2007). Rapid mixing is the rapid dispersal followed by intense agitation of the coagulant into the solution. The optimum rapid mixing retention time is dependent on the velocity gradient coagulant dosage applied. As the velocity gradient and coagulant dosage increases the rapid mixing time will decrease for effective coagulation/flocculation to occur.

Aluminium sulphate ($Al_2(SO_4)_3$) and ferric chloride ($FeCl_3$) are the most commonly used inorganic coagulants (Sarkar *et al.*, 2006; Zhou *et al.*, 2008). These positively charged molecules interact with the negatively charged particles to assist in charge aggregation (Chesters *et al.*, 2009). These coagulants contain trivalent cations and are preferred over divalent cations as coagulation efficiency increases with increased valency (Bratby, 2006). In an aqueous solution the agents are hydrolysed or hydrated to form different manomeric and polymeric species. As pH or coagulant concentration increases the manomeric species are hydrolysed to form various metal hydroxide polymers. These polymers compounds have amorphous structures, with large surface areas, positive charges and hydrophobic properties that favour the interaction with organic particles (Salehizadeh & Shojaosadati, 2001; Dominguez *et al.*, 2005; Gabelich *et al.*, 2006; Zayas *et al.*, 2007; Chesters *et al.*, 2009). The particle destabilisation is brought about by the aluminium and ferric polymers acting as intermediates in the eventual precipitation of the metal hydroxides. Floc formation is a result of subsequent collisions between the smaller particles due to the Brownian motion leading to the formation of larger flocs (Hogg, 2000; Mohana *et al.*, 2008; Zhou *et al.*, 2008).

Chitosan is a modified, natural carbohydrate biopolymer derived from chitin (N-acetyl-2-aminocellulose) (Selmer-Olsen *et al.*, 1996; Lalov *et al.*, 2000). It has cationic properties and is normally used for the recovery of proteinaceous materials, clarifying and recovery of by-products in wastewater (Selmer-Olsen *et al.*, 1996). Chitosan has advantages over inorganic and synthetic coagulation/flocculation agents due to its biodegradability, non-toxic properties and the possibility for regeneration leading to a number of applications (Lalov *et al.*, 2000). Lalov *et al.* (2000) successfully treated vinasse by removing 90% COD using 10 $g.L^{-1}$ chitosan.

There are many different linear and branched polymers with high molecular weights and variable charge densities (Chesters *et al.*, 2009). Anionic polymers become negatively charged when dissolved in water whereas the cationic polymers become positively charged (Chesters *et al.*, 2009). The most popular organic polymers are the high molecular weight polyquaternary cationic amines. The high molecular weight polymers form as coiled chains and when dissolved in water, the charged areas on the chain repel each other leading to the uncoiling of these chains. These polymers are capable of forming large flocs if well mixed by interparticulate bridging thereby achieving increased settling (Bolto *et al.*, 1999; Zhou *et al.*, 2008; Chesters *et al.*, 2009). These polymers present several advantages over inorganic and natural coagulation/flocculation agents: pH independent, lower level of dissolved ions in the treated water, no residual added metals,

smaller sludge production and increased coagulation/flocculation effectiveness when combined with inorganic agents (Bolto *et al.*, 1999; Chesters *et al.*, 2009). Disadvantages of the synthetic polymers include: no clear understanding of exact mechanism, thus making it difficult to optimise the choice of polymer when treating different types of wastewater. Possible health and environmental issues and the possibility of reactions with other chemicals present in wastewater are also factors to be taken into consideration (Bolto *et al.*, 1999).

Sarker *et al.* (2006) treated dairy wastewater with alum (aluminium sulphate) and ferric chloride and found that an increased pH (6.5 – 8.0) increased the effectiveness of dosing. Zhou *et al.* (2008) found that increased dosing while keeping pH constant when treating secondary yeast wastewater increased colour and COD reduction to 90 and 60%, respectively. Al-Mutairi *et al.* (2004) treated slaughterhouse effluent with a combination of an alum salt ($Al_2(SO_4)_3.H_2O$) and a commercially available polymer. The COD and soluble solids (SS) removal ranged from 3 - 20% and 98 - 99%, respectively using an alum salt (100 - 1000 $mg.L^{-1}$) at a pH of 4 - 9 (Al-Mutairi *et al.*, 2004). Using the polymer Al-Mutairi and co-workers removed up to 43% COD and 96% SS.

*Dissolved Air Flotation (DAF)*

Dissolved air flotation is the process whereby particles are separated from water by the addition of small air bubbles that range in size (from 10 to 100 µm). Dissolved air flotation is normally applied where sedimentation techniques are not feasible, due to the presence of extremely fine particles or globules such as oil. The finely suspended particles adhere to the surface of rising bubbles, this increases their buoyancy and allows them to rise to the surface (Al-Shamrani *et al.*, 2001; Zouboulis & Avranas, 2000). Natural hydrophobic materials, such as oil, are ideal candidates for such treatments. Various DAF processes exist and can be summarised as total pressurisation, partial pressurisation and recycle pressurisation. Total pressurisation involves the full pressurisation of the influent and releasing thereof into the flotation tank. This technique is commonly used for wastewaters not requiring flocculation but require large volumes of air bubbles. Partial pressurisation is used for wastewaters where the suspended solids are susceptible to shearing effects of the pressure pump and thus involves the partial pressurisation of the wastewater and directly introducing it into the flotation tanks. Recycled pressurisation is the most commonly used technique for oil containing wastewaters. Between 20 and 50% of the DAF treated effluent is recycled, pressurised and mixed with the effluent. Recycle

pressurisation will incorporate the use of a coagulation/flocculation product (Al-Shamrani *et al*., 2001; Zouboulis & Avranas, 2000).

Al-Shamrani *et al.* (2001) prepared synthetic industrial wastewater containing an oil-in-water emulsion. Treatment of the wastewater consisted of using an aluminium sulphate as a coagulant followed by a DAF treatment. By investigating various operating parameters they were able to achieve near complete oil separation from the synthesised wastewater (Al-Shamrani *et al.,* 2001). Zouboulis and Avranas (2000) followed the same technique of incorporating DAF with an inorganic coagulant (Ferric Chloride) to treat a synthesised oil-in-water emulsion containing wastewater. Various operating parameters were evaluated resulting in the successful separation and removal of more than 95% of the emulsified oil from the wastewater (Zouboulis & Avranas, 2000). Manjunath (1999) evaluated the performance of incorporating an DAF system as pre-treatment and subsequent UASB treatment when treating slaughterhouse effluent. At a ORL of 1.2 $kgCOD.m^{-3}.d^{-1}$ the reactor treating raw effluent was able to maintain an COD removal efficiency ranging from 70 to 75% whereas the UASB reactor treating pre-treated effluent was able to achieve a removal efficiency in the range of 80 to 85% (Manjunath *et al.*, 2000).

*Adsorption*

Adsorption onto materials in order to improve colour and reduce COD of wastewater has been widely adopted. Activated carbon is one of the most broadly employed materials due to its high surface area (600 – 1600 $m^2.g^{-1}$), micro porous structure, high adsorption capacity and high degree of surface reactivity (Droste, 1997; Satyawali & Balakrishnan, 2007). Activated carbon is prepared in such a manner that results in a larger surface area and may be available as a granular (granular activated carbon) or powder (powdered activated carbon) form (Droste, 1997). Different adsorption products are available on the market and include bagasse, bagasse flyash, saw dust, wood ash, rice husks and chitosan (Lalov *et al.*, 2000; Satyawali & Balakrishnan, 2007). Lalov *et al.* (2000) experimented with chitosan to treat distillery wastewater. Treating the wastewater with chitosan at a concentration of 10 $g.L^{-1}$ and a contact time of 30 minutes, Lalov *et al.* (2000) was able to reduce the COD by 93%. Treating biomethanated distillery wastewater using activated carbon Satyawali *et al.* (2007) was able to reduce COD and colour by 83 and 80%, respectively (Satyawali & Balakrishnan, 2007).

*Ozone treatment*

Studies have shown ozone ($O_3$) treatment to be an effective treatment option for wastewater of different origins containing hazardous contaminants such as dyes, phenolics, organochlorides, ammonium compounds and pesticides (Beltran *et al.*, 2001; Sreethawong & Chavadej, 2007; Rivas *et al.*, 2009). Ozone is a powerful oxidising agent as well as a biocide and is a promising alternative to conventional techniques of oxidation and disinfection (Droste, 1997). Ozone is soluble in water thus making it readily available to instantly react with any organic compounds present (Sreethawong & Chavadej, 2007). The versatility of ozone is based on destroying contaminants via two routes, either by direct molecular attack or decomposing into hydroxide ($OH^-$) radicals (Rivas *et al.*, 2009). The partial oxidation of certain compounds can be advantageous if required, however, a subsequent treatment is a necessity if further treatment is required. Ozone treatment becomes a costly procedure (from installing and high electricity consumption) if used in combination with other treatments (Droste, 1997; Sreethawong & Chavadej, 2007).

Different types of hybrid treatment techniques have been explored by various researchers. The use of an integrated ozonation and aerobic digestion treating cherry stillage increased BOD and COD reduction to 85 and 95%, respectively (Beltran *et al.*, 2001). Lee *et al.* (2008) used a DOF (dissolved ozone flotation) system treating municipal wastewater and achieved 81% TSS reduction and 82.4% BOD reduction as well as obtaining a 100% disinfection efficiency. Streethawog and Chavadej (2007) made use of iron oxide ($Fe_2O_3$), a heterogeneous catalyst, to enhance the oxidation of distillery wastewater and subsequently improving the ozone treatment efficiency. The COD reduction improved from 25 (without $Fe_2O_3$) to 63% ($Fe_2O_3$) (Sreethawong & Chavadej, 2007). Green (2007) evaluated the effect of pre-ozonation on wetland efficiency treating winery distillery wastewater (WDWW). The COD reduction improved from 62 to 73% treating a low COD WDWW (2 200 $mg.L^{-1}$) and from 78 to 84% treating high COD WDWW (7 000 $mg.L^{-1}$) (Green, 2007). Green (2007) also reported improved reduction of polyphenols, colour, total solids, soluble solids and phosphates. The COD reduction improved from 78 to 84% (Green, 2007). Gie (2007) investigated the efficiency of a UASB reactor treating WDWW in combination with either a pre- and/or post-ozonation step. The use of a pre- or post-ozonation step during UASB treatment of WDWW resulted in a COD reduction of 94 and 96%, respectively. Combining the UASB treatment with a pre- and post-ozonation step improved the COD reduction to 98% (Gie, 2007).

## AEROBIC BIOLOGICAL TREATMENT

*Enzymatic Treatment*

Increased COD in wastewater might prevent the efficiency of a biological treatment, especially if any microorganisms are inhibited during digestion. Thus, a necessity arises to remove as much of the COD as possible prior to a biological treatment (Cammarota & Freire, 2006). The purpose of enzymatic treatment is to hydrolyse the organic matter, thereby accelerating the process of degradation and the time required for treatment (Cammarota & Freire, 2006; Mendes *et al.*, 2006). Although a costly procedure, enzymatic treatments present advantages such as: applicability to biorefractory compounds, absence of shock loading effects; no biomass generation; absence of delays associated with acclimatisation of the biomass; operation over a wide range of pH, temperature and salinity and ease of controlling the process (Sangave & Pandit, 2006a; Sangave & Pandit, 2006b; Valladão *et al.*, 2007). Sangave and Pandit reported an increased COD reduction (18 – 29%) when a 12 h enzymatic pre-treatment step was included during the aerobic treatment of distillery wastewater. Lipase shows promise being produced by a variety of organisms especially for the treatment of FOG-rich wastewater, similar to GDWW. In addition to the hydrolysing effect of lipase, it decreases the suspended solids and improves colour removal (Mendes *et al.*, 2006). A jar batch experiment conducted by Valladão *et al.* (2007) treating poultry slaughterhouse wastewater combined a pre-hydrolysis step with an anaerobic digestion step. Valladão *et al.* (2007) used a 0.1% enzymatic solution as pre-hydrolysis step followed by anaerobic digestion to improve the COD reduction and biogas production from 53% and 37 mL to 85% and 175 mL, respectively, after 4 days treatment. Higher biogas production and COD reduction (78.2%) were also observed by Mendes *et al.* (2006) when a 12h lipase pre-treatment was followed by anaerobic digestion during the treatment of lipid-rich dairy wastewater (Mendes *et al.*, 2006).

*Lagoon technology*

Also known as wastewater stabilisation ponds, lagoon treatment has been successfully used for primary, secondary and tertiary treatment of different types of wastewater (Maynard *et al.*, 1999; Steinmann *et al.*, 2003). The low operation and maintenance costs involved and little need for specialised skills to operate a lagoon has led to this type of technology being widely adopted across the world during the last century (Maynard *et al.*, 1999; Nataraj *et al.*, 2006). Treatments may either be aerobic, facultative or anaerobic

(Gavrilescu, 2002). Souring during periods of inactivation, contamination of groundwater when using unlined systems and the amount of land required to treat large volumes of wastewater can create problems if a single treatment option is used (Maynard *et al.*, 1999; Mohana *et al.*, 2008). The use of lagoon post-treatment after a conventional treatment to polish the wastewater by removing pathogenic bacteria before discharging have been well documented (Maynard *et al.*, 1999). Rao (1972) employed two anaerobic lagoons when treating distillery wastewater resulting in increased BOD reduction ranging from 82 to 92%.

*Land application*

Soil has a substantial capacity to treat and assimilate organic wastes (Khaleel *et al.*, 1981). Land treatment involves the controlled application of wastes onto the land surface to achieve a specified level of treatment. Treatment can occur through natural physical, chemical or biological processes within the water – soil – plant matrix (Crites *et al.*, 2001; Hati *et al.*, 2007). The unsaturated flow achieved by intermittent dosages results in an extensive contact between wastewater constituents and the soil matrix. These systems can achieve a high purification efficiency due to the complex interactions of hydraulic and purification processes (Van Cuyk *et al.*, 2001). This treatment can be subdivided into three types: Slow rate, overland flow and rapid infiltration (Crites *et al.*, 2001). Increased organic matter – soil aggregation promotes water holding capacity and decreases erosion. The cost effectiveness of this system has led to it being adopted worldwide and when applied to crops it may act as a source of plant nutrients. Hati *et al.* (2007) applied distillery effluent, rich in organic matter and plant nutrients (potassium and sulphur), to arable land as irrigation water and found an increase in crop yield. Applying waste to land at agronomic rates for plant nutrient supplementation has been a traditional means of waste management, however, the interest has shifted to waste disposal in excess of the traditional management (Khaleel *et al.*, 1981). There has also been concern for potential odour development, dissolution of salts in the soil and discharging of hazardous constituents into the groundwater (Crites *et al.*, 2001).

*Wetlands*

Wetlands have been used since the 1950's for the treatment and purification of wastewater (Verhoeven & Meuleman, 1999; Johansson *et al.*, 2004). These semi-aquatic systems can be divided into two groups, natural and constructed wetlands. Lake marginal wetlands, extensive fenland systems, floodplain marshes and tidal freshwater areas are examples of natural wetland systems (Verhoeven & Meuleman, 1999; Pell *et al.*, 2008).

Subsurface flow, surface flow, free water surface and infiltration wetlands are examples of constructed wetlands. Water flows horizontally over the wetland sediment in a surface flow wetland while wastewater is allowed to flow vertically through highly permeable sediment in an infiltration wetland (Verhoeven & Meuleman, 1999). These systems contain populations of emergent plants, helophytes, which were either deliberately planted or naturally colonised the area (Johansson *et al.*, 2004). Successful treatments of domestic, municipal and industrial wastewater using a wetland system have been recorded, however, wetlands are mainly used as a polishing step following a conventional treatment. The performance of a wetland system equates to the loading rate, hydrological and ecological properties of these systems (Verhoeven & Meuleman, 1999). The treatment of distillery wastewater by wetland application has emerged as a simple, cost effective and self-sustaining alternative to conventional treatments especially for the removal of toxicants including heavy metals (Singh *et al.*, 2005; Mohana *et al.*, 2008). Billore *et al.* (2001) was able to successfully treat distillery effluent using a four celled constructed wetland. The first two cells were used for a pre-treatment step, *Typha latifolia* and locally grown *Phragmites karka* were, respectively, planted in cell three and four. The combined treatment of these cells insured 64% COD, 85% BOD, 42% TSS and 79% phosphate reduction, respectively. Singh *et al.* (2005) studied the bioremediation potential of *Potamogeton pectinatus* treating distillery effluent with regards to the bioaccumulation and toxicity of heavy metals. The aquatic macrophyte *P. pectinatus* was able to bioaccumulate heavy metals (Fe, Cu, Zn and Mn) and effectively treat distillery wastewater (Singh *et al.*, 2005). Green (2007) studied the effect of pre-ozonation on the efficiency of a wetland system treating wine distillery wastewater. By combining a pre-ozonation step with a subsequent wetland treatment the COD reduction efficiency increased from 62 to 73% at a COD load of 2 200 mg $COD.L^{-1}$ (Green, 2007). The effectiveness of this treatment was also shown when efficiency increased from 78 to 84% at a COD load of 7 000 mg $COD.L^{-1}$ (Green, 2007).

*Activated Sludge systems*

Activated sludge treatment systems were introduced during 1914 by Arden and Lockett and have ever since shown to be an efficient system for treating a variety of different wastewaters (Sponza, 2002; Pell *et al.*, 2008; Al-Mutairi, 2009). The technology is based on the growth of a microbial population when treating wastewater. A large portion of the treated waste is converted to new cells (McCarty, 1964). A heterogeneous composition of filamentous microorganisms, organic and inorganic polymers, metabolic excretions and

EPS (extracellular polymeric substances) lead to floc development (Sponza, 2002). The effectiveness of the process can be ascribed to good solids – liquid separation ability of the clarifier. The clarifier in-turn can also be influenced by the biological and physicochemical properties of the flocs (Sponza, 2002). Excess sludge production due to rapid growth is an inevitable drawback of the system. The excess sludge must be disposed of and this may account for up to 60% of the total operating costs of the treatment (McCarty, 1964; Ucisik & Henze, 2008). Other disadvantages of using an activated sludge system includes the production of turbid effluents and unpleasant odours, low density flocs, high costs of operation and filamentous bulking (Sponza, 2002; Al-Mutairi, 2009). Torrijos *et al.* (1997) treated winery wastewater generated from a small winery using an aerobic activated sludge system. Up to 93% COD and 97% BOD reduction was achieved (Torrijos & Moletta, 1997). Treatment of wine distillery wastewater (WDWW) using an aerobic activated sludge system at 25ºC improved COD reduction from 31% (HRT = 24h) to 85% (HRT = 72h) (Benitez *et al.*, 2003).

*Sequencing batch reactor*

Sequencing batch reactors (SBR) have been shown to be a highly efficient, simply operated process for the treatment of municipal, industrial and agricultural wastewater (Brenner *et al.*, 2000; Alvarez, 2007). This suspension biomass technology operation is based on batch steps instead of a continuous process, and these steps can be summarised as follows: Feed, react, settle and decant (Alvarez, 2007; Bergamo *et al.*, 2009). The changeable cyclic phasing creates a flexible process for efficient nutrient removal and desired effluent quality (Kang *et al.*, 2003; Bergamo *et al.*, 2009). Effluent quality depends on sludge settlability and a reliable decanting facility (Bae *et al.*, 2003). A single reaction vessel is used for treatment and clarification thus saving space as well as investment costs. The modifications of cycles and phase times creates a flexible process and hazardous wastes in each batch can be tested before decanting (Cassidy *et al.*, 2000; Alvarez, 2007). Potential disadvantages may include poor clarification and turbid effluent as well as increased complexity when using multiple reaction vessels (Cassidy *et al.*, 2000; Kang *et al.*, 2003).

Sequencing batch reactors may either be used as a single treatment step or in combination with other treatment techniques. A membrane separation based sequencing batch reactor was used to treat dairy industry wastewater. A successful treatment was achieved with 97% BOD reduction, 96% nitrogen (N) reduction and 80% phosphorous (P) reduction. Membrane treatment assured a soluble, solids-free effluent (Bae *et al.*, 2003).

Andreottola *et al.* (2002) treated winery wastewater with a sequencing batch biofilm reactor and achieved COD reduction efficiency of between 86 and 99% at a loading rate of *ca.* 8.8 $kgCOD.m^{-3}.d^{-1}$. Up to 70% of the colour was removed with a SBR treating distillery wastewater (Shayegan *et al.*, 2005).

*Trickling Filters*

Work done by researchers showed the potential of trickling filters as a possible option to treat various types of industrial and municipal wastewater (Kamstra *et al.*, 1998; Evangelho *et al.*, 2001; Eding *et al.*, 2006; Kornaros & Lyberatos, 2006; Travieso *et al.*, 2006). Trickling filters are the most commonly used fixed film treatment and present several advantages such as high process stability due to constant high oxygen ($O_2$) levels, carbon dioxide ($CO_2$) effectively removed by degassing, a low energy requirement, the simplicity in design, construction and management (Evangelho *et al.*, 2001; Eding *et al.*, 2006). The trickling filter consists of a fixed media bed through which wastewater would trickle down across the height of the filter (Eding *et al.*, 2006). Microorganisms are attached to a solid substratum in which they reach relatively high concentrations (Evangelho *et al.*, 2001). Wastewater thus flows down over a thin aerobic biofilm and dissolved substrate would diffuse into the biofilm, and thus as the water trickles downwards the water is constantly oxygenated while $CO_2$ is degassed. Typically used substratum includes stones, ceramic material, coal or plastic. The substratum must have a high surface area to allow higher yields of microorganisms making it possible to retain microorganisms with a slow growth rate (Evangelho *et al.*, 2001). Although an efficient treatment option for low strength wastewater the trickling filter has a relatively low volumetric removal rate, making it unsuitable for high strength wastewater. Biofilm shedding may also take place and if the system is not properly designed or operated, a risk of clogging may exist (Eding *et al.*, 2006). Travieso *et al.* (2006) treated distillery wastewater using a combined anaerobic–aerobic filter trickling system to achieve 80% COD reduction, this treatment was followed by a tertiary treatment using a stabilisation pond to increase the COD reduction to 94%.

*Rotating biological contactors*

Rotating biological contactors (RBC) are a proven technology for the large scale application of wastewater treatment (Guimarães *et al.*, 2005). The system exploits the advantages of both fixed film and suspended growth systems (Costley & Wallis, 2001). It is based on a microbial biofilm that develops on the surface of discs mounted on a horizontal

shaft with at least 40% of the discs submerged in the wastewater at any given time. The rotating discs results in alternating contact between the disc, wastewater and air that allows for aerobic growth (Malandra *et al.*, 2003). Although requiring a high initial capital cost the rotating biological contactor presents positive features such as low operation and maintenance costs, simple process control, short start-up, high biomass concentration, insensitive to toxic shock loadings and effective oxygenation with little sloughing of biomass (Costley & Wallis, 2001; Malandra *et al.*, 2003). Aerobic rotating biological reactors have limited performance when treating high strength wastewaters due to excess sludge production leading to oxygen transfer limitations. The development of the anaerobic rotating biological reactor is a feasible process for the treatment of high strength wastewater (Lu *et al.*, 1995). Malandra *et al.* (2003) used a RBC to treat winery wastewater and found that naturally occurring microorganisms could reduce up to 43% COD with a HRT of 1 h. The use of certain yeasts in a RBC treating synthetic wastewater resulted in up to 95% COD reduction under aerated conditions (HRT of 24 h) (Malandra *et al.*, 2003).

## ANAEROBIC BIOLOGICAL TREATMENT

### *Anaerobic Digestion*

Anaerobic digestion distinguishes itself from aerobic digestion with the catabolic processes occurring in the absence of oxygen (Gerardi, 2003). Anaerobic biological treatment has been used globally for the treatment of a variety of wastewaters due to the development of high rate anaerobic processes and the rising costs in aerobic treatment systems (Beltran *et al.*, 2001). Since the end of the 19$^{th}$ century anaerobic technology has been used for the treatment of household wastes (Gavrilescu, 2002; Chernicharo, 2007). The biological conversion of a significant portion of the organic pollutants in the wastewater into a small percentage of biomass and large percentage of biogas, consisting mainly of methane ($CH_4$) and carbon dioxide ($CO_2$), are the main goals of anaerobic digestion (Gavrilescu, 2002; Gerardi, 2003). Anaerobic digestion presents several advantages over aerobic treatments such as low energy requirements, low nutrient requirements, ability to operate under high organic loading rates, the storage of acclimated sludge over long periods of time without deterioration and the small land area required (Lettinga *et al.*, 1980b; Forday & Greenfield, 1983; Deepak, 1998; Aiyuk *et al.*, 2006). During Anaerobic digestion organic matter is primarily converted into biogas (60 – 80%), microbial biomass (5 – 15%) and non-degraded material (10 – 30%). In contrast, during aerobic digestion organic matter is

incorporated as microbial biomass (50 – 60%) and $CO_2$ (40 – 50%) (Gavrilescu, 2002; Walsdorff *et al.*, 2005; Chernicharo, 2007). Although anaerobic digestion is a proven method of wastewater treatment initial start-up may be time consuming depending on the type of wastewater treated. The process is very sensitive to operational factors which adversely affect anaerobic digestion performance. These factors include temperature, pH, alkalinity, nutrition, retention time, biomass availability, mixing and upflow velocities and control parameters (Lettinga *et al.*, 1980a; Forday & Greenfield, 1983; Gerardi, 2003; Chernicharo, 2007; Gie, 2007).

*Microbiology of anaerobic digestion*

Anaerobic digestion can be considered as a natural process, representing an accurately balanced ecological system of microorganisms, where different populations have different capabilities and specialised functions. These organisms all work together to degrade organic waste by means of several consecutive biochemical steps into intermediates and finally methane gas, an excellent source of energy (McCarty, 2001; Gavrilescu, 2002; Chernicharo, 2007). The breakdown of these complex organic compounds can be considered a two-step process. In the first stage hydrolytic bacteria are responsible for hydrolysis and fermentation of the organic polymers (carbohydrates, proteins and lipids) into smaller soluble molecules, which may include volatile fatty acids, carbon dioxide and hydrogen gas (Forday & Greenfield, 1983; Chernicharo, 2007). During the second stage organic acids and hydrogen are converted to methane and carbon dioxide by methanogens, strictly anaerobic prokaryotes (Chernicharo, 2007).

Although anaerobic digestion is generally considered a two-step process, it may be subdivided into several steps according to the different consortium of microorganisms responsible (Batstone *et al.*, 2002; Gavrilescu, 2002; Chernicharo, 2007). Fig 2.1 represents the metabolic pathways associated with anaerobic digestion. It is important that an interlinkage between these different groups of microorganism must be obtained in order for the degradation steps to proceed efficiently and without any disturbances (Gavrilescu, 2002). During the hydrolysis stage, acidogenic (or hydrolytic) bacteria are

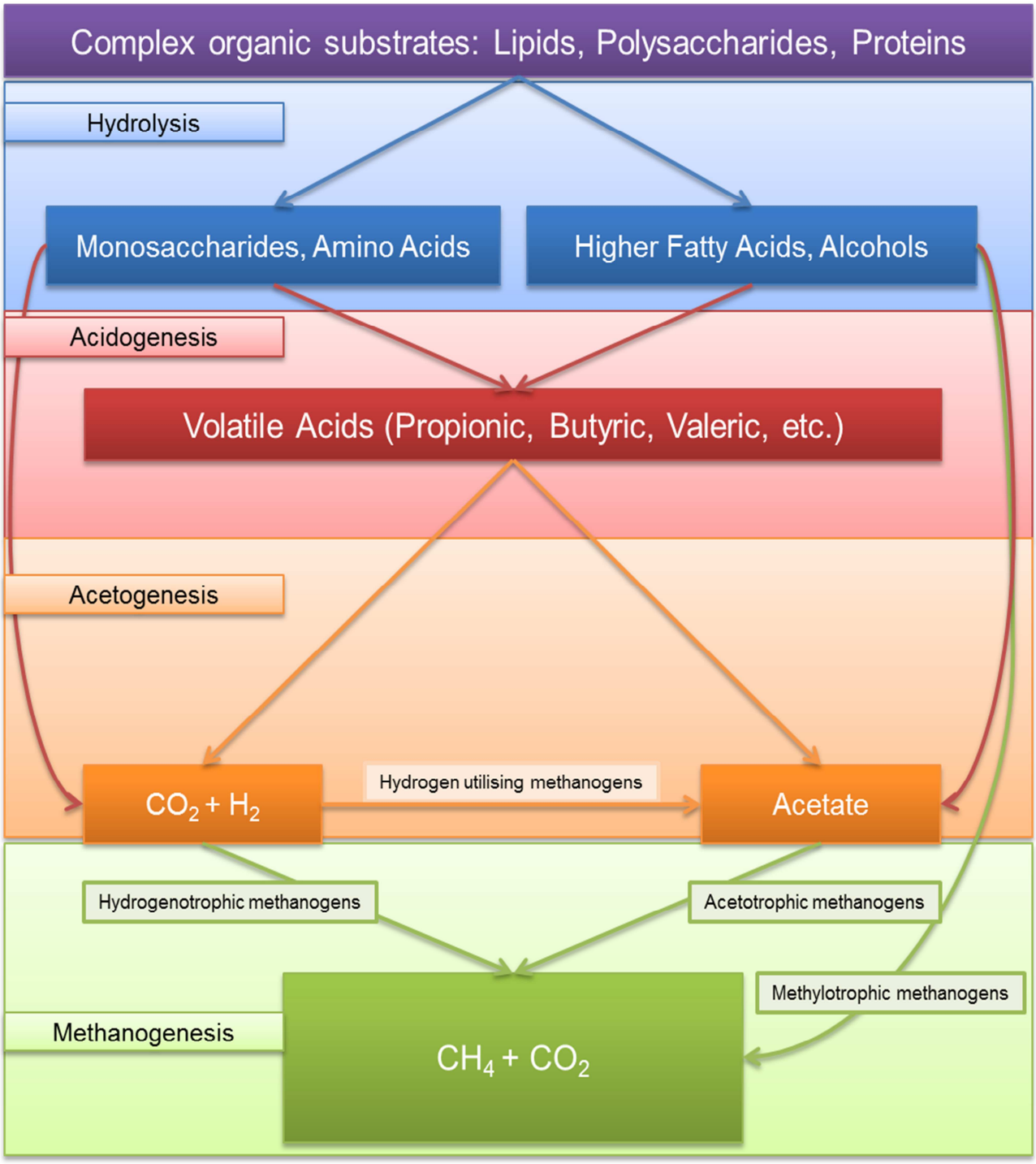


**Figure 2.1** A schematic diagram of anaerobic digestion, indicating the steps involved in the process (adapted from: Forday & Greenfield, 1983; Batstone *et. al.*, 2002; Chernicharo, 2007).

responsible for hydrolysing the particulate organic material (carbohydrates, proteins and lipids) into several simpler soluble compounds (sugars, amino acids and fatty acids) (Forday & Greenfield, 1983; Batstone *et al.*, 2002; Gavrilescu, 2002; Chernicharo, 2007). Acidogens are the largest population of all the different groups and they have a large substrate range and short generation time. This group consists of obligate (*Bacteriodes, Clostridia* and *Bifidobacteria*) and facultative anaerobic (*Streptococci* and *Enterobacteriaceae*) organisms (Forday & Greenfield, 1983; Sahm, 1984). Different exo-enzymes are produced by the acidogens, these enzymes are released by the cells and solubilise the particulate and colloidal substrates, these substrates can enter the cells where they are further degraded by endo-enzymes (Gerardi, 2003; Chernicharo, 2007). The production of these enzymes is a slow process under anaerobic conditions, thus optimal conditions must be sustained at all times to ensure effective degradation of insoluble organic material (Batstone *et al.*, 2002; Gerardi, 2003; Chernicharo, 2007). Soluble products formed during hydrolysis enter the cells of the fermentative bacteria, where they can be further degraded into several simpler compounds including volatile fatty acids (VFA), alcohols, organic acids, carbon dioxide, hydrogen, ammonia and hydrogen sulphide (Gerardi, 2003; Chernicharo, 2007).

Acetogenic bacteria (acetogens) are mainly responsible for the formation of the appropriate substrates for methanogenic bacteria (methanogens). Intermediates such as organic acids, alcohols and organic nitrogen compounds are degraded to acetate by the acetogens. With the formation of the appropriate substrates, hydrogen tends to accumulate leading to a decrease in pH in the aqueous medium. Acetogens are extremely sensitive and can only survive in very low concentrations of hydrogen, thus hydrogen needs to be consumed in order to avoid any disturbances (Forday & Greenfield, 1983). Methanogens, homo-acetogens and sulphate reducing bacteria can utilise hydrogen during the formation of methane and the formation of propionic and butyric acid can lower the partial hydrogen pressure (Gavrilescu, 2002; Gerardi, 2003; Chernicharo, 2007). The successful conversion of VFA's is important, because the unionised form of these acids are toxic to the methanogens (Forday & Greenfield, 1983). Homo-acetogens contribute to acetic acid formation by carbohydrate degradation, they are also able to consume hydrogen but their significance is considered to be minor as they are unable to effectively compete with the methanogens (Forday & Greenfield, 1983; Batstone *et al.*, 2002).

During methanogenesis the specific products (acetic acid, hydrogen, carbon dioxide, formic acid, methanol, methylamines and carbon monoxide) formed during the digestion stages are converted to methane and carbon dioxide by methanogens

(Chernicharo, 2007). The microorganisms are obligate anaerobes and are often considered the key class of microorganisms in anaerobic digestion (Walsdorff *et al.*, 2005). All methanogens can grow autotrophically on hydrogen and carbon dioxide as sole energy and carbon source (Forday & Greenfield, 1983; Aiyuk *et al.*, 2006). Methanogens can be divided into three groups according to their affinity to any of the substrates and includes hydrogenotrophic methanogens, acetotrophic methanogens and methylotrophic methanogens. Hydrogenotropic methanogens are responsible for the conversion of carbon dioxide into methane and maintaining a low partial hydrogen pressure (Gerardi, 2003). These organism are also responsible for the conversion of formate to methane (Aiyuk *et al.*, 2006).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$
$$4CO + 2H_2O \rightarrow CH_4 + 3CO_2$$

Acetotrophic methanogens are the established microorganisms in anaerobic digestion and are responsible for between 60 and 70% of all the methane production taking place (Aiyuk *et al.*, 2006). Acetotrophic methanogens are slow growing (2.6 days) organisms which are also affected by the accumulated hydrogen, and effective reduction of hydrogen is also favourable to these methanogens as well as to acetogens (Gerardi, 2003; Chernicharo, 2007).

$$4CH_3COOH \rightarrow 4CO_2 + 2H_2$$

Methylotrophic methanogens can produce methane from substrates containing a methyl group, these include methanol and methylamines (Gerardi, 2003).

$$3CH_3OH + 6H \rightarrow 3CH_4 + 3H_2O$$
$$4(CH_3)_3 - N + 6H_2O \rightarrow 9CH_4 + 3CO_2 + 4NH_3$$

*High Rate Reactors*

Increased usage and development of anaerobic digestion technology has led to the successful implementation of high rate anaerobic bioreactors. These anaerobic reactors can be characterised by the achievable organic loading rate (Deepak, 1998; Akunna & Clark, 2000). Increased solids retention and low hydraulic retention time results in a reactor capable of reaching a high organic loading rate without compromising the valuable

biomass through sludge immobilisation (Barber & Stuckey, 1999; Rajeshwari *et al.*, 2000; Cavaleiro *et al.*, 2001; Gavrilescu, 2002). Slow growth rates of anaerobic bacteria compared to aerobic systems may be challenging, as any disturbances such as changes in wastewater characteristics, presence of toxic compounds and changes in pH and temperature, will be harmful to the system and recovery may take much longer in anaerobic systems, especially high rate reactors (Rajeshwari *et al.*, 2000; Cavaleiro *et al.*, 2001). Thus, it is very important that a thorough understanding of the system is needed to steer clear of any problems and achieve proper treatment of wastewater (Cavaleiro *et al.*, 2001). High rate anaerobic reactors may be classified into 3 main groups based on their mechanism to achieve effective biomass retention. These groups include fixed film, suspended growth and hybrid bioreactors (Barber & Stuckey, 1999; Rajeshwari *et al.*, 2000).

Anaerobic Baffled Reactor

An anaerobic baffled reactor (ABR) is a high rate reactor in a single reactor configuration, with compartments. These compartments let the system enjoy the additional benefits of a multiphase reactor without the related problems and operational cost requirements (Barber & Stuckey, 1999; Saritpongteeraka & Chaiprapat, 2008). The compartments enable the system to retain biomass within the reactor for longer periods of time (high solids retention time) independent of the hydraulic retention time (Saritpongteeraka & Chaiprapat, 2008). In addition the compartments will also enable the reactor to separate the acidogens from the methanogens longitudinally down the reactor and thus increase the reduction efficiency (Barber & Stuckey, 1999). Boopathy *et al.* (1988) successfully treated distillery effluent; with an organic loading rate of 3.5 $kgCOD.m^{-3}.day^{-1}$ they achieved a reduction efficiency of 91% over an operational period of 210 days. Palm oil mill wastewater was treated using a modified ABR - COD reduction varied between 87 and 95% whereas grease/oil reduction ranged between 44 and 91% (Faisal & Unno, 2001).

To overcome the difficulties of substrate characteristics and the accumulation of VFA's within the system, extensive research has led to the development of the split fed anaerobic baffled reactor (SFABR) (Uyanik, 2003; Mohana *et al.*, 2008; Mohana *et al.*, 2009) . This system reduces the severity of the conditions created by the wastewater in the initial compartments and allows increased mixing within. Uyanik (2003) was able to successfully treat distillery wastewater at a COD loading rate at 10.5 $kgCOD.m^{-3}.d^{-1}$ with a SFABR system achieving 90% COD reduction after 70 days.

Granular Bed Anaerobic Baffled Reactor (GRABR)

At a low hydraulic retention time problems may occur within a UASB system due to the low density of the granules and washout may take place if appropriate measures are not taken. Fluctuations in wastewater flow parameters may also interrupt reactor efficiency due to uneven distribution or channelling of wastewater within the reactor (Akunna & Clark, 2000). Akunna and Clark (2000) proposed developing a GRABBR, an anaerobic baffled reactor coupled with the UASB concept for the treatment of high strength wastewater. They found that the system could effectively treat whiskey distillery wastewater at an OLR of 4.75 kgCOD.$m^{-3}$.$day^{-1}$ with a COD reduction efficiency of up to 80%.

Fixed Bed Reactor

Also known as an anaerobic filter or fixed film reactor the system contains a microbial supporting material (Gavrilescu, 2002; Chaisri *et al.*, 2007). Granules not only exist within the spaces of the support material but also become immobilised by attachment onto the material, thus leading to a higher microbial density and possibly higher reduction efficiencies (Alves *et al.*, 2001a; Chaisri *et al.*, 2007). Contact between wastewater and microorganisms takes place by the upflow movement through the fixed material (Cavaleiro *et al.*, 2001). Increased performance can thus be related to support material properties, surface area, type of material used and reactor parameters (Cavaleiro *et al.*, 2001). One of the major advantages of the fixed bed reactor is the ability of the system to retain a SRT at a low HRT. Sludge washout is also unlikely to occur with the physical protection of the support material (Alves *et al.*, 2001a; Gavrilescu, 2002; Aiyuk *et al.*, 2006). The system is only suitable for treating low soluble solids concentrations and plugging may develop with high organic loading rate conditions. The possibility of channelling and clogging within the system makes it difficult to judge the quantity and quality of the biomass (Alves *et al.*, 2001a; Gavrilescu, 2002). Using a two stage system consisting of an anaerobic filter and a UASB reactor Blonskaja *et al.* (2003) successfully treated distillery wastewater at loading rates of 5.1 and 2.5 kgCOD.$m^{-3}$.$d^{-1}$ between the 1$^{st}$ and 2$^{nd}$ stages, respectively, and reached reduction efficiencies of 93% overall.

## D. UPFLOW ANAEROBIC SLUDGE BLANKET (UASB) REACTOR

The UASB reactor was developed and introduced by Lettinga *et al.* (1980) and has since become one of the most popular and versatile high rate anaerobic treatment systems throughout the world. The UASB system presents an attractive solution because of low

operational cost, low energy consumption, compact design, low sludge production and production of methane, a potential energy source (Lettinga *et al.*, 1980b; Forday & Greenfield, 1983; Goodwin *et al.*, 1990; Chernicharo, 2007). Successful treatment of a wide variety of different wastes including sugar industry wastes, distillery wastes and brewery wastes has led to more than a 1 000 UASB units being adopted by different industries all over the world (Droste, 1997; Gavrilescu, 2002; Chernicharo, 2007).

The UASB reactor operates as a suspended growth system, active biomass is held in suspension by hydraulic design without the use of any packing material (Deepak, 1998; Gavrilescu, 2002; Tiwari *et al.*, 2006). The success of the UASB reactor depends on granule formation (Gavrilescu, 2002; Tiwari *et al.*, 2006; Mohana *et al.*, 2008). These granules are the result of the aggregation of anaerobic bacteria, making them more resistant to shock loadings and toxicity. The granular biomass presents several advantages over dispersed cells: microorganisms are densely grouped, no inert support medium enables the maximum use of reactor volume, the spherical form of the granules enables a maximum microorganism to volume ratio and the granules present excellent settleability (Chernicharo, 2007). Granules can be up to 5 mm in diameter with excellent settleable properties enabling a higher hydraulic loading rate (Goodwin *et al.*, 1990; Gavrilescu, 2002; Mohana *et al.*, 2008). Each granule can be described as a spherical biofilm consisting of different groups of anaerobic bacteria, each group playing a role during the degradation of wastewater (Tiwari *et al.*, 2006). The cultivation of a good quality biomass is achieved through a careful start-up of the process, during which the artificial selection of the biomass is imposed, allowing the poor quality sludge to be washed out of the system while retaining the good quality sludge (Chernicharo, 2007).

## OPERATIONAL PRINCEPLES OF AN UASB REACTOR

The UASB reactor (Fig. 2.2) consists of four major parts including the granular sludge bed, sludge blanket, gas-solids separator and a settlement compartment (Droste, 1997; Mohana *et al.*, 2008). The sludge bed and sludge blanket is composed of granules, the biomass responsible for the degradation of the wastewater. Wastewater enters the reactor at the bottom as influent and treatment occurs once contact has been achieved between the granules and the wastewater. Production of biogas causes internal circulation and mixing within the sludge bed creating more efficient granule wastewater contact (Gavrilescu, 2002; Chernicharo, 2007).

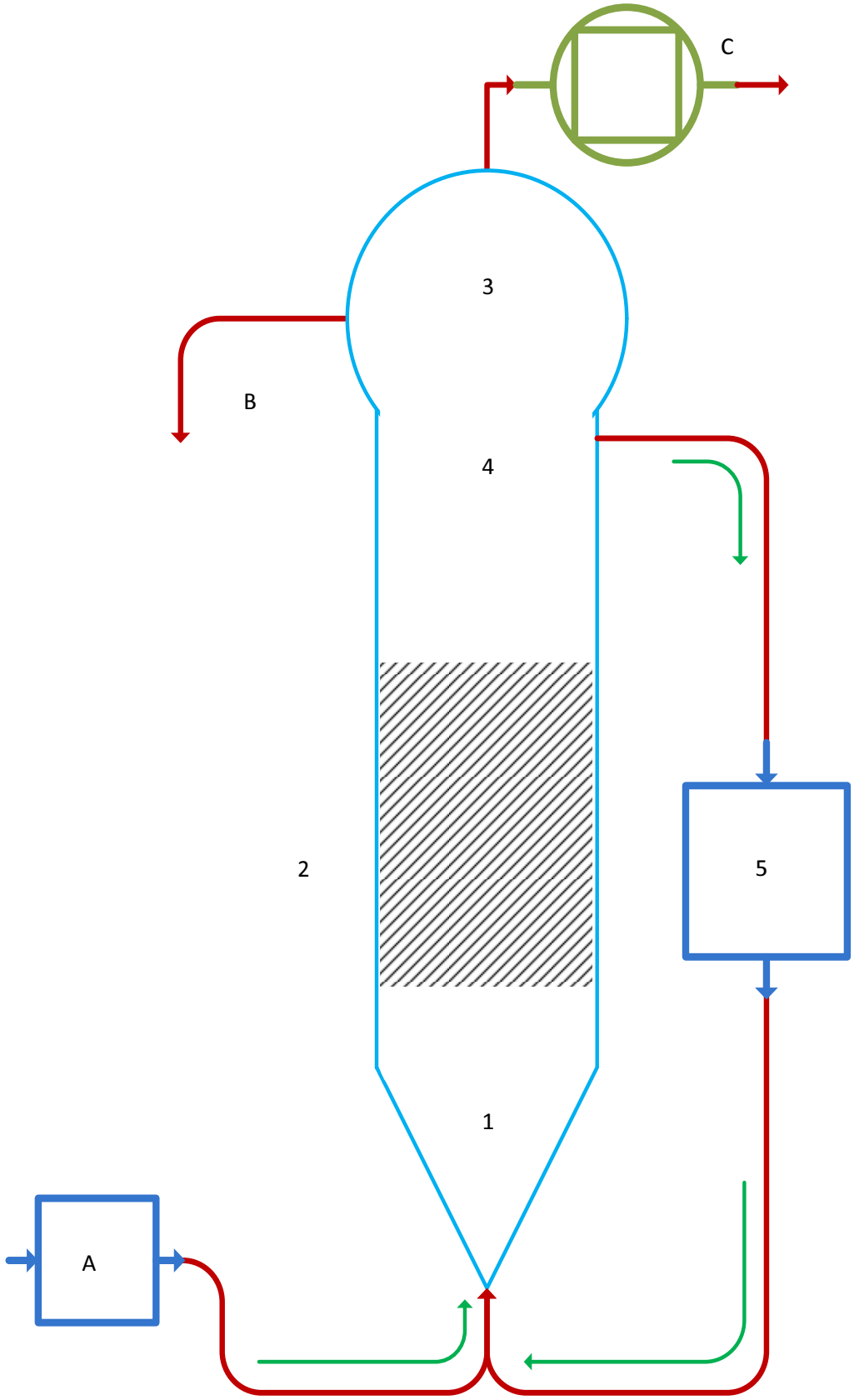


**Figure 2.2** A schematic representation of an UASB reactor: (1) granular sludge bed; (2) sludge blanket; (3) gas solids separator; (4) settlement compartment; (5) recirculation pump; (A) influent; (B) effluent and (C) gas outlet (Schmidt & Ahring, 1996; Gavrilescu, 2002; Chernicharo, 2007).

The upward movement of gas bubbles and liquid flow automates mixing of the biomass throughout the sludge bed and sludge blanket. The gas-solid separator (GSS) is located at the top end of the reactor and ensures sludge retention while gas escapes through the outlet (Chernicharo, 2007). A small amount of granules and floc may enter the settlement compartment. In this inactive zone the granules may either settle back into the reactor or be washed out as effluent (Schmidt & Ahring, 1996).

## EXTRINSIC FACTORS GOVERNING ANAEROBIC DIGESTION AND UASB REACTOR EFFICIENCY

*Nutrients*

Biological treatment processes like the UASB process require macronutrients such as the organic material to sustain growth and carry out the biochemical transformations. In addition to the requirement of macronutrients a number of the microorganisms involved in anaerobic digestion require some essential micronutrients for metabolism, growth, activity and stabilisation of the UASB process (Singh *et al.*, 1999). Industrial wastewaters, like GDWW, are more specific in composition than municipal wastewater. Thus, for optimum growth and performance of bacteria it is important to provide additional nutrients to the system which will result in better reactor treatment (McCarty, 1964). Two important macronutrients to consider in biological treatments include nitrogen (N) and phosphorus (P) (Gerardi, 2003). Phosphorous is required for cell maintenance and cell growth (Singh *et al.*, 1999). Ammonia can serve as a source of nitrogen, electron donor and buffer. All anaerobic bacteria can utilise nitrogen and it has been shown that nitrogen supplementation increased the activity of methanogens (Tiwari *et al.*, 2006). However, a build-up of nitrogen in the system may also lead to alterations in intracellular pH resulting in the inhibition of methane synthesising enzymes (Singh *et al.*, 1999; Tiwari *et al.*, 2006). Research has shown that a combination of nitrogen, phosphorous and potassium supplementation prevents the negative effects of shock loadings and the prevention of sludge wash out (Singh *et al.*, 1999; Tiwari *et al.*, 2006). Treatment of high strength wastewaters in a UASB reactor requires a C:N:P ratio of 1000:7:1 (Sahm, 1984; Gerardi, 2003).

In addition to the requirement of macronutrients a number of anaerobic organisms, especially methanogens require some essential micronutrients. These nutrients are essential for bacterial growth, activity and subsequent efficiency of the UASB reactor and are required in very small amounts (Singh *et al.*, 1999). Trace elements essential to

anaerobic digestion include iron ($Fe^{2+}$), aluminum ($Al^{3+}$), cobalt ($Co^{3+}$), nickel ($Ni^{2+}$), sulfide ($S^{2-}$), magnesium ($Mg^{2+}$), calcium ($Ca^{2+}$) and manganese ($Mn^{2+}$) (Singh *et al.*, 1999; Gerardi, 2003; Tiwari *et al.*, 2006). Methanogens possess several unique enzymes that require certain trace elements, whose introduction will contribute to more efficient methane production (Singh *et al.*, 1999; Tiwari *et al.*, 2006). Studies done by Goodwin (1990) suggested that nutrients play an important role during anaerobic digestion. If any mineral or trace element were removed from the system a decrease in performance by either acidogens or methanogens was observed. Re-introducing the specific nutrients led to greater reduction efficiencies and increased digester performance. Other researchers have shown that although nutrient supplementation can increase methanogenic activity they also increases the acidogenic activity. Increased gas production is accompanied by an increase in reactor pH due to increased methanogenic activity, but increase in acidogenic activity will lower the pH within the reactor (Kim *et al.*, 2002).

*Temperature*

Two optimum temperatures can be considered when using a UASB reactor, these include mesophilic (30 – 35°C) and thermophilic (50 – 55°C) conditions (McCarty, 1964). A constant temperature has to be maintained throughout the reactor to prevent any variations or undesirables within the reactor that may lead to decreased performance (Gerardi, 2003). The use of thermophilic anaerobic treatment shows several advantages over mesophilic reactors such as increased rate of organic matter degradation, improved solids–liquid separation and increased destruction of pathogenic organisms (Kim *et al.*, 2002). Although increased digestion is proportional to an increased rate of biogas production several problems have been encountered with thermophilic digestion (Gerardi, 2003). These problems may include: yield of microorganisms being lower under thermophilic conditions, a higher death rate (double that of mesophilic reacto), a lack in diversity and inconsistency during treatment of wastewater (Kim *et al.*, 2002; Gerardi, 2003; Tiwari *et al.*, 2006). Increased concentrations of VFA may also be encountered due to the limited bioavailability of nutrients under these conditions (Kim *et al.*, 2002).

At a decreased operating temperature biological activity is increased related to cell retention time and mesophilic conditions provide a faster and more stable start-up compared to thermophilic sludge (Tiwari *et al.*, 2006; Bergamo *et al.*, 2009). Any sudden changes in temperature will have a negative impact on reactor performance. Borja *et al.* (1995) has shown that a sudden decrease in temperature was characterised by an immediate decrease in pH below the normal operating levels. The decreased pH led to a

sudden increase in effluent VFA's. After stabilisation of the reactor's temperature the effluent VFA levels began to decrease.

*pH and alkalinity*

Control of pH is one of the most important environmental factors governing an efficient and stable reactor, and a UASB system performs well between a pH of 6.6 and 7.6 (McCarty, 1964; Wentzel *et al.*, 1994; Droste, 1997). The preservation of the pH in an anaerobic digester is a result of the interaction of the weak acid/base system present (Wentzel *et al.*, 1994). The weak acid/base buffering against pH change are the carbonates (characterised by $H_2CO_3$ alkalinity and pH). The main weak acid/base causing pH decline are the short chain fatty acids (SCFA) (Wentzel *et al.*, 1994). The maintenance of this near neutral pH is due to the conversion of acid end products (SCFA) to methane by the combined activities of acetogens and methanogens (Forday & Greenfield, 1983). The sensitivity of anaerobic digestion reflects the sensitivity of methanogens and any rapid pH changes within the reactor can be toxic, thus accurate control must be maintained to favour the conditions for methanogens (Forday & Greenfield, 1983; Moosbrugger *et al.*, 1993). Degradation of the wastewater leads to intermediates such as volatile acids and carbon dioxide, these products lead to a decrease in system pH and the system is required to accommodate any changes in pH (Moosbrugger *et al.*, 1993; Droste, 1997).

Alkalinity serves as a buffer in the system, it prevents any rapid pH changes (Gerardi, 2003; Saritpongteeraka & Chaiprapat, 2008). The carbonate – bicarbonate system is the controlling buffer regulating the system pH with orthophosphoric acid, hydrosulfuric acid, VFA's and ammonia contributing to pH stabilisation (Forday & Greenfield, 1983). The acidogenic phase dominates the lower part of the bed leading to an increase in SCFA produced which reduces the alkalinity and pH (Wentzel *et al.*, 1994). In the upper part of the bed the SCFA are converted to $CH_4$ and $CO_2$, and alkalinity is regenerated (Wentzel *et al.*, 1994). Reactor stability can be correlated to a high alkalinity value and any decrease below the norm (1 000 – 3 000 $mg.L^{-1}$) may be due to the inability of the methanogens to successfully convert organic acid (Wentzel *et al.*, 1994; Gerardi, 2003).

*Retention time*

Two significant retention times in an anaerobic digester are solids retention time (SRT) and hydraulic retention time (HRT) (Speece, 1983; Gerardi, 2003). The SRT is the average time that bacteria are in the anaerobic digester. A maximum SRT (>12 days) is

desirable for process stability due to the slow generation time of certain organisms, like methanogens (Sahm, 1984; Gerardi, 2003). A high SRT is advantageous to an anaerobic digester: it maximises the COD reduction capacity and reduces the digester volume required. Furthermore, a high SRT increases the buffering capacity for protection against shock loadings and promotes the acclimatisation to toxic compounds (Gerardi, 2003).

The HRT controls the conversion of volatile solids to gaseous products in an anaerobic digester and should be long enough to ensure that sufficient degradation occurs (Gerardi, 2003). A low HRT minimises the reactor volume and thus reduces the required capital cost (Speece, 1983). Droste (1997) suggested a HRT of less than 24h as adequate when designing a digester to treat distillery wastewater.

*Biomass immobilisation*

Biomass immobilisation can be achieved by either attachment to a fixed (anaerobic filters) or moving solid supporters (fluidized bed reactors) (Alves *et al.*, 1999). UASB reactors operate without the use of any support material, the upflow provides a constant selective pressure on the microorganism so they can start adhering to each other leading to better settleability (Deepak, 1998; Gavrilescu, 2002; Tiwari *et al.*, 2006; Chernicharo, 2007). Achieving biomass (granules) immobilisation is important for efficient anaerobic digestion. Successful biomass immobilisation will result in increased kinetics of degradation and increased resistance to inhibitory intermediates (Lettinga *et al.*, 1997; Chernicharo, 2007).

*Mixing and upflow velocity*

Mixing enhances the digestion process by distributing microorganisms, substrates and nutrients uniformly throughout the digester as well as equalising the temperature throughout (Gerardi, 2003; Karim *et al.*, 2005). Mixing in the reactor aids in particle size reduction as digestion occurs as well as the removal of gas from the mixture (Karim *et al.*, 2005). Upflow velocity and biogas load both contribute to the selective washout of lighter, non-granulating biomass. Aggregates or granules improve the overall stability of the digester. Severe upflow velocities may lead to bed washout or disintegration of granules (Karim *et al.*, 2005).

## OPERATIONAL PROBLEMS EXPERIENCED DURING ANAEROBIC DIGESTION

The UASB reactor has been shown to be an effective treatment option for various types of wastewater, however, reports have shown that problems may occur if the system is not

designed correctly and monitored effectively (Akunna & Clark, 2000). During large scale operations suitable flow distribution may be difficult to achieve and maintained and this problem may be aggravated by fluctuations in wastewater flow. This can lead to decreased reactor efficiency and poor effluent quality (Akunna & Clark, 2000).

Variations in reactor parameters (temperature, OLR and start-up) or the presence of toxicants can lead to kinetic uncoupling between the acid producers and consumers followed by accumulation in VFA's (Mechichi & Sayadi, 2005). Accumulation of VFA's is followed by a decrease in alkalinity and lowering of the system pH (Gerardi, 2003). Stable performance of an anaerobic reactor greatly depends on the establishment of a suitable microbial consortium during start-up (Rossini *et al.*, 1999; McMahon *et al.*, 2004). It was found that during quick start-ups, butyrate, propionate and acetate accumulated when treating sewage sludge. This accumulation led to an overall decreased digester performance (Griffin *et al.*, 1998). Several workers have reported on the difficulty of treating olive mill waste water, with instability and poor reproducibility occurring in UASB reactor start-ups (Rossini *et al.*, 1999; Franceschi *et al.*, 2002).

## TREATMENT OF FOG – RICH WASTEWATER

*Treatment difficulties of FOG-rich wastewater*

High strength wastewater like GDWW is rich in biodegradable organic molecules, nutrients and rich in fats, oils and grease (FOG). The GDWW also contains proteins having low biodegradability coefficients. If not treated correctly these constituents that make up GDWW can cause pollution to land, water and treatment systems (Mendes & Castro, 2005; Cammarota & Freire, 2006). If FOG cannot be retained in the pre-treatment systems and enters the biological system it can become a nuisance (Cammarota & Freire, 2006). Several problems are associated with lipids during biological treatment.

The formation of a lipid coating around the floc can cause several problems within both aerobic and anaerobic treatment systems. In aerobic systems the reduction of oxygen transfer can severely hamper the efficiency of the microorganisms leading to a loss in system performance (Cavaleiro *et al.*, 2001; Cammarota & Freire, 2006). The increase in filamentous microorganisms may cause further problems during pumping and aeration of the system that can lead to the formation of scum that hinders biomass flocculation and sedimentation (Laubscher *et al.*, 2001; Cammarota & Freire, 2006). The adsorbed lipid's specific gravity and the inability of the sludge to settle will lead to sludge bed wash out in anaerobic systems such as UASB reactors (Cavaleiro *et al.*, 2001; Hwu, 2001; Chipasa &

Mdrzycka, 2008). Hwu (2001) reported that sludge flotation is directly proportional to the loading rate and that the time required for complete bed washout decreases with a higher loading rate. Laubscher *et al.* (2001) experienced operational problems when treating GDWW in a UASB reactor, a foam layer manifested as a highly gelatinous layer which interfered with the effluent overflow and in some cases it was so harsh that it halted the operation.

The anaerobic metabolism of lipids involves several steps. Lipids are hydrolysed to form glycerol and long chain fatty acids (LCFA). Biodegradability of LCFA increases with a decreasing carbon chain length and increased degree of unsaturation (Batstone *et al.*, 2002; Chipasa & Mdrzycka, 2008). The LCFA are then further degraded via the β-oxidation pathway to acetic acid and hydrogen by proton reducing acetogens (Alves *et al.*, 2001b; Cavaleiro *et al.*, 2001; Pereira *et al.*, 2002). Thus, lipid degradation using a microbial consortium takes a long time. Various workers have reported the inhibitory effects caused by LCFA on acetoclastic methanogens (acetic acid utilizing methanogenic bacteria) and hydrogen producing acetogens (responsible for LCFA oxidation) (Koster & Cramer, 1987; Mendes & Castro, 2005; Miranda *et al.*, 2005; Cammarota & Freire, 2006; Chipasa & Mdrzycka, 2008). Although the mechanism of LCFA inhibition is not completely understood the inhibitory effect increases with the number of double bonds and cis-isomers (Cammarota & Freire, 2006). Gram positive microorganisms including methanogens, are more susceptible than Gram negative organisms to LCFA (Koster & Cramer, 1987). A decrease in reduction efficiency will be followed by a decrease in methane concentration ($CH_4$), decrease in pH and an increase in VFA levels, which can result in reactor failure (Cavaleiro *et al.*, 2001). However, under practical conditions, complete bed washout is likely to occur before microbial inhibition (Chipasa & Mdrzycka, 2008).

*Methane potential of FOG-rich wastewater*

Lipids are an attractive substrate for biogas production for their high potential methane yield capacity compared to proteins and carbohydrates and can be considered as a potential renewable energy source (Cirne *et al.*, 2007; Sousa *et al.*, 2007). Cirne *et al.* (2007) investigated the effects of lipid concentration, ranging from 5 to 47% (w/w), on the hydrolysis and biomethanation of FOG-rich (triolein) wastewater. With an initial lag phase experienced methane recovery, as a result of slow degradation of compounds, methane recovery increased to above 93% at all concentrations (Cirne *et al.*, 2007). However,

removal of lipids during pre-treatments owing to their associated problems have led to a loss of this potential energy (Sousa *et al.*, 2007).

*Enhancing the treatment effectiveness of FOG-rich wastewater*

Although lipids have proven to be troublesome during aerobic and anaerobic biological treatments studies have shown that these systems can adapt to lipid-rich wastewater under a strict feeding strategy, thus enhancing the methane production in anaerobic treatment. Anaerobic digestion of LCFA-rich wastewater is possible, provided that a continuously-fed, well mixed digester is used and sudden overloading is avoided (Rinzema *et al.*, 1994). Design of the digester must account for the types of wastewater to be treated. The slow degradability and potential inhibitory effect of LFCA must be accounted for (Lalman & Bagley, 2000). The gradual replacement of a substrate with lipids during a dosing can increase the resistance of methanogens and acetogens to LCFA toxicity (Alvarez, 2007; Cavaleiro *et al.*, 2007; Neves *et al.*, 2007). Cavaleiro *et al.* (2007) promoted the development of a specialised anaerobic community by cycles of increased loading rate feeds to efficiently degrade a mixture of skim milk and sodium oleate. Goncalves *et al.* (2011a) compared acclimatised sludge to unnacclimatised sludge treating olive mill wastewater. Acclimatised sludge showed more resistance as well as experiencing no lag phase during the start-up of the experiment, whereas unacclimatised sludge experienced a lag phase due to the inhibition of acetoclastic methanogens.

The requirement of acclimatisation might turn out to be the bottleneck of FOG-rich wastewater treatment because of the slow growth rate of LCFA-degraders and the limited source of LCFA-/FOG-adapted biomass. Thus, for full-scale anaerobic treatment the introduction of FOG-rich wastewater should start at a low concentration to allow for acclimatisation and retention of microorganisms capable of FOG degradation (Hwu, 2001).

*UASB treatment of FOG-rich wastewater and GDWW*

Successful treatments of different types of wastewaters by UASB systems have been documented by researchers. For the treatment of grain distillery wastewater Gao *et al.* (2007) successfully used a UASB system and achieved up to 97.3% COD reduction at an OLR between 5 and 48 $kgCOD.m^{-3}.d^{-1}$. Goodwin (1994) found that only after diluting, adjustment of pH and settling could malt whisky wastewater be treated successfully at a stable OLR of no higher than 15 $kgCOD.m^{-3}.d^{-1}$, with an increased loading rate leading to system instability occurring subsequently followed by reactor failure. Enhancement of the UASB process, by combining the treatment with other technologies, is the apparent choice

to achieve successful treatment of this type of wastewater. The use of two stage systems has also been well documented. Borja & Banks (1995) suggested using a two stage UASB system for treatment of palm oil mill effluent after a single stage became unstable when the OLR passed 10 kgCOD.$m^{-3}$.$d^{-1}$. Using a two stage system consisting of a dissolved air flotation system (DAF) and UASB system, COD reduction of up to 90% was achieved when treating slaughterhouse effluent at OLR of 4 kgCOD.$m^{-3}$.$d^{-1}$ (Manjunath *et al.*, 2000). Uzal *et al.* (2003) achieved COD reduction of up to 96% using a two stage UASB system when treating malt whisky wastewater and anaerobic treatment was followed by aerobic treatment to further increase the COD reduction to 99.5%. Jeganathan *et al.* (2006) treated pet food wastewater containing high amounts of oils and grease with a novel hybrid packed bed reactor – upflow anaerobic sludge bed (PBR-UASB) reactor. The PBR was packed with alginate beads containing immobilised lipase responsible for hydrolysis of the oils and grease before entering the UASB reactor (Jeganathan *et al.*, 2006). Jeganathan and co-workers achieved 41% hydrolysis at a oils and grease loading rate of 0.9 kgCOD.$m^{-3}$.$d^{-1}$ in the PBR and subsequently 90% O&G reduction overall efficiency for the PBR-UASB reactor.

The expanded granular sludge bed (EGSB) reactor is a modification of the UASB reactor and present several advantages (Dinsdale *et al.*, 2000). The granules are partially fluidised by effluent recycle at high upflow velocities (5-6 m.$h^{-1}$) and have been applied in situations where the volumetric gas production rate is low and mixing in a UASB reactor is inefficient. The system also allows a higher OLR than a UASB system and prevents the gas-piston effect often experienced in lab-scale UASB reactors at a high volumetric gas loading rate(Dinsdale *et al.*, 2000).

## D. DISCUSSION

Distilleries can be classified as a high polluting industry due to the nature of the wastewater produced during processing (Nataraj *et al.*, 2006; Mohana *et al.*, 2008; Sowmeyan & Swaminathan, 2008). The industry is rapidly expanding to meet the ever increasing demand worldwide. One form of distillery wastewater is GDWW, a complex wastewater rich in organic material and FOG, a by-product of whisky production. GDWW can result in severe environmental implications if left untreated (Mendes & Castro, 2005; Cammarota & Freire, 2006).

Several treatments are in use today, each efficient at removing excess solids, colloidal material and, more importantly, FOG from wastewater. A coagulation/flocculation

treatment is an important physico-chemical treatment in reducing these particulates from wastewater. Several parameters have to be considered that will influence coagulation/flocculation efficiency. These parameters include solution pH, type of coagulant/flocculant used, dosages applied, mixing retention times and eventual disposal of waste after treatment. By employing a coagulation/flocculation as pre-treatment sufficient amounts of FOG and solids can be removed from GDWW before subsequent treatment can commence. Little research up to now has been done on the effect of such a pre-treatment on reducing FOG in GDWW and the subsequent influence it will have on the effectiveness of an UASB reactor treating GDWW.

The UASB reactor has become a popular and versatile anaerobic treatment system and presents an attractive solution because of low operation costs, low energy consumptions, compact design, low sludge production and the production of methane gas(Lettinga *et al.*, 1981; Forday & Greenfield, 1983; Goodwin *et al.*, 1990). Although successful treatments of distillery wastewater have been documented the complexity of GDWW presents treatment problems to anaerobic treatment systems such as the UASB reactor. Adsorbed lipids onto the biomass can result in sludge bed washout (Cavaleiro *et al.*, 2001; Hwu, 2001; Chipasa & Mdrzycka, 2008). Formation of LCFA during FOG metabolism can have a inhibitory effect on the microbial consortia responsible for breakdown of organic matter (including FOG) during treatment, thus indirectly influencing the UASB reactor efficiency (Koster & Cramer, 1987; Mendes & Castro, 2005; Miranda *et al.*, 2005; Cammarota & Freire, 2006; Chipasa & Mdrzycka, 2008).

In order to successfully treat GDWW in a UASB reactor, it is suggested that a pre-treatment is applied to remove sufficient amounts of solids and FOG. This may be accomplished by incorporating a coagulation/flocculation treatment. Once solids and, more importantly, FOG have been reduced the chances of acclimatising the anaerobic consortia to the wastewater should be increased. The effect of pre-treatment and subsequent effect on reactor efficiency would still need to be investigated.

## E. REFERENCES

Adewumi, J., Ilemobade, A. & Van Zyl, J. (2010). Treated wastewater reuse in South Africa: overview, potential and challenges. *Resources, Conservation and Recycling*, **55**, 221-231.

Agu, R., Bringhurst, T. & Brosnan, J. (2006). Production of grain whisky and ethanol from wheat, maize and other cereals. *Journal of the Institute of Brewing*, **112**, 314-323.

Agu, R., Bringhurst, T., Brosnan, J. & Jack, F. (2008). Effect of Process Conditions on Alcohol Yield of Wheat, Maize and Other Cereals. *Journal of the Institute of Brewing*, **114**, 39-44.

Aguilar, M.I., Sáez, J., Lloréns, M., Soler, A. & Ortuño, J.F. (2002). Nutrient removal and sludge production in the coagulation-flocculation process. *Water Research*, **36**, 2910-2919.

Aiyuk, S., Forrez, I., Lieven, K., Van Haandel, A. & Verstraete, W. (2006). Anaerobic and complementary treatment of domestic sewage in regions with hot climates—A review. *Bioresource Technology*, **97**, 2225-2241.

Akunna, J.C. & Clark, M. (2000). Performance of a granular-bed anaerobic baffled reactor (GRABBR) treating whisky distillery wastewater. *Bioresource Technology*, **74**, 257-261.

Al-Mutairi, N.Z. (2009). Aerobic selectors in slaughterhouse activated sludge systems: A preliminary investigation. *Bioresource Technology*, **100**, 50-58.

Al-Mutairi, N.Z., Hamoda, M.F. & Al-Ghusain, I. (2004). Coagulant selection and sludge conditioning in a slaughterhouse wastewater treatment plant. *Bioresource Technology*, **95**, 115-119.

Alvarez, J.A., Lema, J., Barros, S., Lara, E. (2007). Treatment of distillery wastewaters (vinasses) by an anaerobic sequential batch reactor in pilot plant. In: *11th IWA World Congress on Anaerobic Digestion*. 1 - 6. Brisbane, Australia.

Alves, M., Mota Vieira, J., Alvares Pereira, R., Pereira, M. & Mota, M. (2001a). Effect of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors. Part I: biofilm growth and activity. *Water Research*, **35**, 255-263.

Alves, M., Vieira, J., Pereira, R., Pereira, M. & Mota, M. (2001b). Effects of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors. Part II: Oleic acid toxicity and biodegradability. *Water Research*, **35**, 264 - 270.

Alves, M.M., Pereira, M.A., Novais, J.M., Polanco, F.F. & Mota, M. (1999). A New Device to Select Microcarriers for Biomass Immobilization: Application to an Anaerobic Consortium. *Water Environment Research*, **71**, 209-217.

Anonymous (2009). What is whisky. [WWW document] Wine direct. [[URL] www.winedirectonline.co.za]. 10/09/2009.

APHA (1998). *Standard methods for the examination of water and wastewater*, 20th ed. Pp. 2:26-28, 22:54-58, 24:86-87, 24:149-150, 145:113-117, 145:134-139. Washington D. C.

Bae, T.-H., Han, S.-S. & Tak, T.-M. (2003). Membrane sequencing batch reactor system for the treatment of dairy industry wastewater. *Process Biochemistry*, **39**, 221-231.

Barber, W.P. & Stuckey, D.C. (1999). The use of the anaerobic baffled reactor (ABR) for wastewater treatment: a review. *Water Research*, **33**, 1559-1578.

Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S., Pavlostathis, S., Rozzi, A., Sanders, W., Siegrist, H. & Vavilin, V. (2002). The IWA Anaerobic Digestion Model No 1(ADM 1). *Water Science & Technology*, **45**, 65-73.

Beltran, F., Alvarez, P., Rodriguez, E., Garcia-Araya, J. & Rivas, J. (2001). Treatment of high strength distillery wastewater (cherry stillage) by integrated aerobic biological oxidation and ozonation. *Biotechnology progress*, **17**.

Benitez, F.J., Real, F.J., Acero, J.L., Garcia, J. & Sanchez, M. (2003). Kinetics of the ozonation and aerobic biodegradation of wine vinasses in discontinuous and continuous processes. *Journal of Hazardous Materials*, **101**, 203-218.

Bergamo, C.M., Di Monaco, R., Ratusznei, S.M., Rodrigues, J.A.D., Zaiat, M. & Foresti, E. (2009). Effects of temperature at different organic loading levels on the performance of a fluidized-bed anaerobic sequencing batch bioreactor. *Chemical Engineering and Processing: Process Intensification*, **48**, 789-796.

Boller, M.A. & Kavanaugh, M.C. (1995). Particle characteristics and headloss increase in granular media filtration. *Water Research*, **29**, 1139-1149.

Bolto, B., Abbt-Braun, G., Dixon, D., Eldridge, R., Frimmel, F., Hesse, S., King, S. & Toifl, M. (1999). Experimental evaluation of cationic polyelectrolytes for removing natural organic matter from water. *Water Science & Technology*, **40**, 71-79.

Brenner, A., Shandalov, S., Messalem, R., Yakirevich, A., Oron, G. & Rebhun, M. (2000). Wastewater reclamation for agricultural reuse in Israel: Trends and experimental results. *Water, Air, & Soil Pollution*, **123**, 167-182.

Cammarota, M.C. & Freire, D.M.G. (2006). A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresource Technology*, **97**, 2195-2210.

Cassidy, D.P., Efendiev, S. & White, D.M. (2000). A comparison of CSTR and SBR bioslurry reactor performance. *Water Research*, **34**, 4333-4342.

Cavaleiro, A., Alves, J. & Alves, M. (2007). Discontinuous operation promotes efficient continuous anaerobic treatment of effluents with high lipid content. In: *11th IWA World Congress on Anaerobic Digestion*. Brisbane, Australia.

Cavaleiro, A.J., Alves, M.M. & Mota, M. (2001). Microbial and operational response of an anaerobic fixed bed digester to oleic acid overloads. *Process Biochemistry*, **37**, 387-394.

Chaisri, R., Boonsawang, P., Prasertsan, P. & Chaiprapat, S. (2007). Effect of organic loading rate on methane and volatile fatty acids productions from anaerobic treatment of palm oil mill effluent in UASB and UFAF reactors. *Songklanakarin Journal of Science and Technology*, **29**.

Chernicharo, C.d.L. (2007). *Anaerobic Reactors,* Pp. 1 - 184. IWA Publishing.

Chesters, S., Darton, E., Gallego, S. & Vigo, F. (2009). The safe use of cationic flocculants with reverse osmosis membranes. *Desalination and Water Treatment*, **6**, 144-151.

Chipasa, K.B. & Mdrzycka, K. (2008). Characterization of the fate of lipids in activated sludge. *Journal of Environmental Sciences*, **20**, 536-542.

Cirne, D.G., Paloumet, X., Björnsson, L., Alves, M.M. & Mattiasson, B. (2007). Anaerobic digestion of lipid-rich waste--Effects of lipid concentration. *Renewable Energy*, **32**, 965-975.

Clare, K., Ramatapa, E. & Currin, B. (2004). Study of the Liquor Industry in South Africa. (edited by T. D. o. T. a. Industry). South Africa: Reality Research Africa.

Costley, S.C. & Wallis, F.M. (2001). Bioremediation of heavy metals in a synthetic wastewater using a rotating biological contactor. *Water Research*, **35**, 3715-3723.

Crites, R., Reed, S. & Bastian, R. (2001). Applying treated wastewater to land: Solving water problems. *Biocycle*, **42**, 32-36.

Csar, P. (2009). Pure Single Malt Scotch Whisky. [WWW document] The Making of Whisky. [www.editme.co.za]. 04/03/09.

Deepak, L.J. (1998). High rate anaerobic treatment of industrial wastewater in tropics. *International Journal of Science and Technology*, **3**, 1 - 7.

Dinsdale, R.M., Hawkes, F.R. & Hawkes, D.L. (2000). Anaerobic digestion of short chain organic acids in an expanded granular sludge bed reactor. *Water Research*, **34**, 2433-2438.

Dominguez, J., de Heredia, J., Gonzalez, T. & Sanchez-Lavado, F. (2005). Evaluation of ferric chloride as a coagulant for cork processing wastewaters. Influence of the operating conditions on the removal of organic matter and settleability parameters. *Industrial and Engineering Chemistry Research*, **44**, 6539-6548.

Droste, R. (1997). *Theory and practice of water and wastewater treatment*. Wiley.

du Plessis, J. (2003). Maize production. [WWW document] Department of Agriculture and ARC Grain Crops Institute, Republic of South Africa. [www.nda.agric.za].

Eding, E.H., Kamstra, A., Verreth, J.A.J., Huisman, E.A. & Klapwijk, A. (2006). Design and operation of nitrifying trickling filters in recirculating aquaculture: A review. *Aquacultural Engineering*, **34**, 234-260.

Evangelho, M.R., Gonçalves, M.M.M., Sant'Anna, G.L. & Villas Bôas, R.C. (2001). A trickling filter application for the treatment of a gold milling effluent. *International Journal of Mineral Processing*, **62**, 279-292.

Faisal, M. & Unno, H. (2001). Kinetic analysis of palm oil mill wastewater treatment by a modified anaerobic baffled reactor. *Biochemical Engineering Journal*, **9**, 25-31.

Fan, L., Xu, N., Ke, X. & Shi, H. (2007). Numerical simulation of secondary sedimentation tank for urban wastewater. *Journal of the Chinese Institute of Chemical Engineers*, **38**, 425-433.

Forday, W. & Greenfield, P.F. (1983). Anaerobic digestion. *Effluent and Water Treatment Journal*, **23**, 405 - 409.

Franceschi, M., Girou, A., Carro-Diaz, A.M., Maurette, M.T. & Puech-Costes, E. (2002). Optimisation of the coagulation–flocculation process of raw water by optimal design method. *Water Research*, **36**, 3561-3572.

Gabelich, C.J., Ishida, K.P., Gerringer, F.W., Evangelista, R., Kalyan, M. & Suffet, I.H.M. (2006). Control of residual aluminum from conventional treatment to improve reverse osmosis performance. *Desalination*, **190**, 147-160.

Gao, M., She, Z. & Jin, C. (2007). Performance evaluation of a mesophilic (37 °C) upflow anaerobic sludge blanket reactor in treating distiller's grains wastewater. *Journal of Hazardous Materials*, **141**, 808-813.

Gavrilescu, M. (2002). Engineering concerns and new developments in anaerobic waste-water treatment. *Engineering concerns and new developments in anaerobic waste-water treatment*, **3**, 346-362.

Gerardi, M.H. (2003). *The Microbiology of Anaerobic Digesters,* Pp. 1 - 192. Wiley.

Gie, L. (2007). Effect of pre-treated distillery wastewater on upflow anaerobic sludge blanket granules and reactor efficiency. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Gogate, P.R. (2002). Cavitation: an auxiliary technique in wastewater treatment schemes. *Advances in Environmental Research*, **6**, 335-358.

Gonze, E., Commenges, N., Gonthier, Y. & Bernis, A. (2003). High frequency ultrasound as a pre- or a post-oxidation for paper mill wastewaters and landfill leachate treatment. *Chemical Engineering Journal*, **92**, 215-225.

Goodwin, J.S., Finlayson, J.M. & Low, E.W. (2001). A further study of the anaerobic biotreatment of malt whisky distillery pot ale using an UASB system. *Bioresource Technology*, **78**, 155-160.

Goodwin, J.S. & Stuart, J.B. (1994). Anaerobic digestion of malt whisky distillery pot ale using upflow anaerobic sludge blanket reactors. *Bioresource Technology*, **49**, 75-81.

Goodwin, J.S., Wase, D.J. & Forster, C.F. (1990). Effects of nutrient limitation on the anaerobic upflow sludge blanket reactor. *Enzyme and Microbial Technology*, **12**, 877-884.

Green, J. (2007). Impact of pre-ozonation on distillery effluent degradation in a constructed wetland system. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Griffin, M., McMahon, K., Mackie, R. & Raskin, L. (1998). Methanogenic population dynamics during start-up of anaerobic digesters treating municipal solid waste and biosolids. *Biotechnology and Bioengineering*, **57**.

Guimarães, C., Porto, P., Oliveira, R. & Mota, M. (2005). Continuous decolourization of a sugar refinery wastewater in a modified rotating biological contactor with Phanerochaete chrysosporium immobilized on polyurethane foam disks. *Process Biochemistry*, **40**, 535-540.

Hati, K.M., Biswas, A.K., Bandyopadhyay, K.K. & Misra, A.K. (2007). Soil properties and crop yields on a vertisol in India with application of distillery effluent. *Soil and Tillage Research*, **92**, 60-68.

Hogg, R. (2000). Flocculation and dewatering. *International Journal of Mineral Processing*, **58**, 223-236.

Hwu, C. (2001). Enhanced anaerobic biological treatment of lipid-containing wastewaters. *Journal of the Chinese Institute ol Environmental Engineering*, **11**, 151-156.

Jayanti, S. & Narayanan, S. (2004). Computational study of particle-eddy interaction in sedimentation tanks. *Journal of environmental engineering*, **130**, 37.

Jeganathan, J., Nakhla, G. & Bassi, A. (2006). Novel hybrid PBR-UASB system for the treatment of high strength pet food wastewater. *Proceedings of the Water Environment Federation*, **2006**, 5040-5048.

Johansson, A.E., Gustavsson, A.M., Öquist, M.G. & Svensson, B.H. (2004). Methane emissions from a constructed wetland treating wastewater--seasonal and spatial distribution and dependence on edaphic factors. *Water Research*, **38**, 3960-3970.

Kamstra, A., van der Heul, J.W. & Nijhof, M. (1998). Performance and optimisation of trickling filters on eel farms. *Aquacultural Engineering*, **17**, 175-192.

Kang, I.-J., Lee, C.-H. & Kim, K.-J. (2003). Characteristics of microfiltration membranes in a membrane coupled sequencing batch reactor system. *Water Research*, **37**, 1192-1197.

Karim, K., Hoffmann, R., Thomas Klasson, K. & Al-Dahhan, M.H. (2005). Anaerobic digestion of animal waste: Effect of mode of mixing. *Water Research*, **39**, 3597-3606.

Khaleel, R., Reddy, K. & Overcash, M. (1981). Changes in soil physical properties due to organic waste applications: A review. *J. Environ. Qual*, **10**, 133-141.

Kim, M., Ahn, Y.-H. & Speece, R.E. (2002). Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. *Water Research*, **36**, 4369-4385.

Kirzhner, F., Zimmels, Y. & Shraiber, Y. (2008). Combined treatment of highly contaminated winery wastewater. *Separation and Purification Technology*, **63**, 38-44.

Kornaros, M. & Lyberatos, G. (2006). Biological treatment of wastewaters from a dye manufacturing company using a trickling filter. *Journal of Hazardous Materials*, **136**, 95-102.

Koster, I. & Cramer, A. (1987). Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids. *Applied and environmental microbiology*, **53**, 403-409.

Kriel, P. (2010). The Nature of Corporate Entreperneurship in the South African Liquor Industry. Gordon Institute of Business Science, University of Pretoria.

Kuang, Y. (2002). Enhancing Anaerobic Degradation of Lipids in Wastewater by Addition of Co-substrate. In: PhD in School of Environmental Science, Murdoch University.

Lalman, J.A. & Bagley, D.M. (2000). Anaerobic degradation and inhibitory effects of linoleic acid. *Water Research*, **34**, 4220-4228.

Lalov, I.G., Guerginov, I.I., Krysteva, M.A. & Fartsov, K. (2000). Treatment of waste water from distilleries with chitosan. *Water Research*, **34**, 1503-1506.

Lapisova, K., Vlcek, R., Klozova, J., Rychtera, M. & Melzoch, K. (2006). Separation techniques for distillery stillage treatment. *Czech Journal of Food Sciences-UZPI (Czech Republic)*.

Lata, K., Kansal, A., Balakrishnan, M., Rajeshwari, K. & Kishore, V. (2002). Assessment of biomethanation potential of selected industrial organic effluents in India. *Resources, Conservation and Recycling*, **35**, 147-161.

Laubscher, A., Wentzel, M., Le Roux, J. & Ekama, G. (2001). Treatment of grain distillation wastewaters in an upflow anaerobic sludge bed (UASB) system. *Water S. A.*, **27**, 433-444.

Lettinga, G., Arnhem, A. & Engineers, C. (1981). Feasibility of the upflow anaerobic sludge blanket (UASB) process for the treatment of low-strength wastes. 625 - 634.

Lettinga, G., Field, J., Van Lier, J., Zeeman, G. & Pol, L. (1997). Advanced anaerobic wastewater treatment in the near future. *Water science and technology*, 5-12.

Lettinga, G., Van Velsen, A., Hobma, S., De Zeeuw, W. & Klapwijk, A. (1980a). Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnology and Bioengineering*, **22**.

Lettinga, G., van Velsen, A.F.M., Hobma, S.W., de Zeeuw, W. & Klapwijk, A. (1980b). Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnology and Bioengineering*, **22**, 699-734.

Lu, C., Yeh, A.C. & Lin, M.-R. (1995). Treatment of high-strength organic wastewaters using an anaerobic rotating biological contactor. *Environment International*, **21**, 313-323.

Malandra, L., Wolfaardt, G., Zietsman, A. & Viljoen-Bloom, M. (2003). Microbiology of a biological contactor for winery wastewater treatment. *Water Research*, **37**, 4125-4134.

Manjunath, N.T., Mehrotra, I. & Mathur, R.P. (2000). Treatment of wastewater from slaughterhouse by DAF-UASB system. *Water Research*, **34**, 1930-1936.

Maynard, H.E., Ouki, S.K. & Williams, S.C. (1999). Tertiary lagoons: a review of removal mecnisms and performance. *Water Research*, **33**, 1-13.

McCarty, P. (1964). Anaerobic waste treatment fundamentals. *Public Works*, **95**, 107-112.

McCarty, P. (2001). The development of anaerobic treatment and its future. *Anaerobic Digestion for Sustainable Development*, **44**, 149-156.

McMahon, K., Zheng, D., Stams, A., Mackie, R. & Raskin, L. (2004). Microbial population dynamics during start-up and overload conditions of anaerobic digesters treating municipal solid waste and sewage sludge. *Biotechnology and Bioengineering*, **87**.

Mechichi, T. & Sayadi, S. (2005). Evaluating process imbalance of anaerobic digestion of olive mill wastewaters. *Process Biochemistry*, **40**, 139-145.

Melamane, X., Strong, P. & Burgess, J. (2007). Treatment of Wine Distillery Wastewater: A Review with Emphasis on Anaerobic Membrane Reactors. *South African Journal for Enology and Viticulture*, **28**, 25.

Mendes, A. & Castro, H. (2005). Effect on the enzymatic hydrolysis of lipids from dairy wastewater by replacing Gum Arabic emulsifier for sodium chloride. *Brazilian Archives of Biology and Technology*, **48**, 135-142.

Mendes, A.A., Pereira, E.B. & de Castro, H.F. (2006). Effect of the enzymatic hydrolysis pretreatment of lipids-rich wastewater on the anaerobic biodigestion. *Biochemical Engineering Journal*, **32**, 185-190.

Miranda, L., Henriques, J. & Monteggia, L. (2005). A full-scale UASB reactor for treatment of pig and cattle slaughterhouse wastewater with a high oil and grease content. *Brazilian Journal of Chemical Engineering*, **22**, 601-610.

Mohana, S., Acharya, B.K. & Madamwar, D. (2008). Distillery spent wash: Treatment technologies and potential applications. *Journal of Hazardous Materials*, **In Press, Corrected Proof**.

Mohana, S., Acharya, B.K. & Madamwar, D. (2009). Distillery spent wash: Treatment technologies and potential applications. *Journal of Hazardous Materials*, **163**, 12-25.

Moosbrugger, R., Wentzel, M., Ekama, G. & Marais, G. (1993). Weak Acid/Bases and pH Control in Anaerobic Systems--A Review. *Water SA WASADV*, **19**.

Nataraj, S.K., Hosamani, K.M. & Aminabhavi, T.M. (2006). Distillery wastewater treatment by the membrane-based nanofiltration and reverse osmosis processes. *Water Research*, **40**, 2349-2356.

Naumann, E. (2005). The South African Liquor Industry. *Department of Trade and Industry*, 1 - 39.

Neves, L., Pereira, M., Mota, M. & Alves, M. (2007). A method to detect and quantify long chain fatty acids in liquid and solid samples and its relevance to understand anaerobic digestion of lipids.

Otieno, F. & Ochieng, G. (2004). Water management tools as a means of averting a possible water scarcity in South Africa by the year 2025. *Water SA*, **30**, 120-124.

Otieno, F. & Ochieng, G. (2007). Water management tools as a means of averting a possible water scarcity in South Africa by the year 2025. *Water SA*, **30**, 120-124.

Pell, M., Wörman, A., Sven Erik, J. & Brian, F. (2008). Biological Wastewater Treatment Systems. In: *Encyclopedia of Ecology*. Pp. 426-441. Oxford: Academic Press.

Pereira, M., Pires, O., Mota, M. & Alves, M. (2002). Anaerobic degradation of oleic acid by suspended and granular sludge: identification of palmitic acid as a key intermediate. *Water Science & Technology*, **45**, 139 - 144.

Perret, S. (2002). Water policies and smallholding irrigation schemes in South Africa: a history and new institutional challenges. *Water Policy*, **4**, 283 - 300.

Piya-Areetham, P., Shenchunthichai, K. & Hunsom, M. (2006). Application of electrooxidation process for treating concentrated wastewater from distillery industry with a voluminous electrode. *Water Research*, **40**, 2857-2864.

Rajeshwari, K.V., Balakrishnan, M., Kansal, A., Lata, K. & Kishore, V.V.N. (2000). State-of-the-art of anaerobic digestion technology for industrial wastewater treatment. *Renewable and Sustainable Energy Reviews*, **4**, 135-156.

Rautenbach, R., Linn, T. & Eilers, L. (2000). Treatment of severely contaminated waste water by a combination of RO, high-pressure RO and NF -- potential and limits of the process. *Journal of Membrane Science*, **174**, 231-241.

Rinzema, A., Boone, M., van Knippenberg, K. & Lettinga, G. (1994). Bactericidal effect of long chain fatty acids in anaerobic digestion. *Water Environment Research*, 40-49.

Ripley, P. (1979). Process engineering aspects of the treatment and disposal of distillery effluent. *Process Biochemistry*, **14**, 8-10.

Rivas, J., Gimeno, O. & Beltrán, F. (2009). Wastewater recycling: Application of ozone based treatments to secondary effluents. *Chemosphere*, **74**, 854-859.

Rossini, M., Garrido, J.G. & Galluzzo, M. (1999). Optimization of the coagulation–flocculation treatment: influence of rapid mix parameters. *Water Research*, **33**, 1817-1826.

Sahm, H. (1984). Anaerobic wastewater treatment. *Advances in Biochemical Engineering/Biotechnology*, **29**, 83-115.

Salehizadeh, H. & Shojaosadati, S.A. (2001). Extracellular biopolymeric flocculants: Recent trends and biotechnological importance. *Biotechnology Advances*, **19**, 371-385.

Sangave, P.C. & Pandit, A.B. (2004). Ultrasound pre-treatment for enhanced biodegradability of the distillery wastewater. *Ultrasonics Sonochemistry*, **11**, 197-203.

Sangave, P.C. & Pandit, A.B. (2006a). Enhancement in biodegradability of distillery wastewater using enzymatic pretreatment. *Journal of Environmental Management*, **78**, 77-85.

Sangave, P.C. & Pandit, A.B. (2006b). Ultrasound and enzyme assisted biodegradation of distillery wastewater. *Journal of Environmental Management*, **80**, 36-46.

Saritpongteeraka, K. & Chaiprapat, S. (2008). Effects of pH adjustment by parawood ash and effluent recycle ratio on the performance of anaerobic baffled reactors treating high sulfate wastewater. *Bioresource Technology*, **99**, 8987-8994.

Sarkar, B., Chakrabarti, P., Vijaykumar, A. & Kale, V. (2006). Wastewater treatment in dairy industries—possibility of reuse. *Desalination*, **195**, 141-152.

Satyawali, Y. & Balakrishnan, M. (2007). Removal of color from biomethanated distillery spentwash by treatment with activated carbons. *Bioresource Technology*, **98**, 2629-2635.

Satyawali, Y. & Balakrishnan, M. (2008). Wastewater treatment in molasses-based alcohol distilleries for COD and color removal: A review. *Journal of Environmental Management*, **86**, 481-497.

SAWIS (2009). South African Wine Industry Statistics. [WWW document] [www.sawis.co.za].

Schmidt, J.E. & Ahring, B. (1996). Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactors. *Biotechnology and Bioengineering*, **43**, 229 - 246.

Selmer-Olsen, E., Ratnaweera, H. & Pehrson, R. (1996). A novel treatment process for dairy wastewater with chitosan produced from shrimp-shell waste. *Water Science & Technology*, **34**, 33-40.

Shayegan, J., Pazouki, M. & Afshari, A. (2005). Continuous decolorization of anaerobically digested distillery wastewater. *Process Biochemistry*, **40**, 1323-1329.

Singh, N., Pandey, G., Rai, U., Tripathi, R., Singh, H. & Gupta, D. (2005). Metal accumulation and ecophysiological effects of distillery effluent on Potamogeton pectinatus L. *Bulletin of Environmental Contamination and Toxicology*, **74**, 857-863.

Singh, R.P., Kumar, S. & Ojha, C.S.P. (1999). Nutrient requirement for UASB process: a review. *Biochemical Engineering Journal*, **3**, 35-54.

Sousa, D., Pereira, M., Alves, J., Smidt, H., Stams, A. & Alves, M. (2007). Anaerobic microbial LCFA degradation in bioreactors.

Sowmeyan, R. & Swaminathan, G. (2008). Effluent treatment process in molasses-based distillery industries: A review. *Journal of Hazardous Materials*, **152**, 453-462.

Speece, R. (1983). Anaerobic biotechnology for industrial wastewater treatment. *Environmental Science & Technology*, **17**, 416-427.

Sponza, D. (2002). Extracellular polymer substances and physicochemical properties of flocs in steady- and unsteady-state activated sludge systems. *Process Biochemistry*, **37**, 983-998.

Sreethawong, T. & Chavadej, S. (2007). Color removal of distillery wastewater by ozonation in the absence and presence of immobilized iron oxide catalyst. *Journal of Hazardous Materials*.

Steinmann, C.R., Weinhart, S. & Melzer, A. (2003). A combined system of lagoon and constructed wetland for an effective wastewater treatment. *Water Research*, **37**, 2035-2042.

Stevenson, D.G. (1997). Flow and filtration through granular media--the effect of grain and particle size dispersion. *Water Research*, **31**, 310-322.

Tiwari, M., Guha, S., Harendranath, C. & Tripathi, S. (2006). Influence of extrinsic factors on granulation in UASB reactor. *Applied Microbiology and Biotechnology*, **71**, 145-154.

Tokuda, M., Ohta, N., Morimura, S. & Kida, K. (1998). Methane fermentation of pot ale from a whisky distillery after enzymatic or microbial treatment. *Journal of Fermentation and Bioengineering*, **85**, 495-501.

Torrijos, M. & Moletta, R. (1997). Winery wastewater depollution by sequencing batch reactor. *Water Science & Technology*, **35**, 249 - 257.

Travieso, L., Sánchez, E., Borja, R., Benítez, F., Raposo, F., Rincón, B. & Jiménez, A.M. (2006). Evaluation of a laboratory-scale stabilization pond for tertiary treatment of distillery waste previously treated by a combined anaerobic filter-aerobic trickling system. *Ecological Engineering*, **27**, 100-108.

Ucisik, A.S. & Henze, M. (2008). Biological hydrolysis and acidification of sludge under anaerobic conditions: The effect of sludge type and origin on the production and composition of volatile fatty acids. *Water Research*, **42**, 3729-3738.

Uyanik, S. (2003). A Novel Anaerobic Reactor: Split Fed Anaerobic Baffled Reactor (SFABR). *Turkish Journal of Engineering Environmental Science*, **27**, 339 -345.

Uzal, N., Gökçay, C.F. & Demirer, G.N. (2003). Sequential (anaerobic/aerobic) biological treatment of malt whisky wastewater. *Process Biochemistry*, **39**, 279-286.

Valladão, A.B.G., Freire, D.M.G. & Cammarota, M.C. (2007). Enzymatic pre-hydrolysis applied to the anaerobic treatment of effluents from poultry slaughterhouses. *International Biodeterioration & Biodegradation*, **60**, 219-225.

Van Cuyk, S., Siegrist, R., Logan, A., Masson, S., Fischer, E. & Figueroa, L. (2001). Hydraulic and purification behaviors and their interactions during wastewater treatment in soil infiltration systems. *Water Research*, **35**, 953-964.

Verhoeven, J. & Meuleman, A. (1999). Wetlands for wastewater treatment: Opportunities and limitations. *Ecological Engineering*, **12**, 5-12.

Walsdorff, A., Van Kraayenburg, M. & Barnardt, C. (2005). A multi-site approach towards integrating environmental management in the wine production industry. *Sustainable Viticulture and Winery Wastes Management*, **51**, 61-69.

Wentzel, M., Moosbrugger, R., Sam-Soon, P., Ekama, G. & Marais, G. (1994). Tentative guidelines for waste selection, process design, operation and control of upflow anaerobic sludge bed reactors. *Water Science & Technology*, **30**, 31-42.

White, D.A. & Verdone, N. (2000). Numerical modelling of sedimentation processes. *Chemical Engineering Science*, **55**, 2213-2222.

Wilkie, A., Riedesel, K. & Owens, J. (2000). Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. *Biomass and Bioenergy*, **19**, 63-102.

Willey, R. (2001). Fats, oils, and greases: The minimization and treatment of wastewaters generated from oil refining and margarine production. *Ecotoxicology and Environmental Safety*, **50**, 127-133.

Williams, G.J., Sheikh, B., Holden, R.B., Kouretas, T.J. & Nelson, K.L. (2007). The impact of increased loading rate on granular media, rapid depth filtration of wastewater. *Water Research*, **41**, 4535-4545.

Zayas, T., Rómero, V., Salgado, L., Meraz, M. & Morales, U. (2007). Applicability of coagulation/flocculation and electrochemical processes to the purification of biologically treated vinasse effluent. *Separation and Purification Technology*, **57**, 270-276.

Zhou, Y., Liang, Z. & Wang, Y. (2008). Decolorization and COD removal of secondary yeast wastewater effluents by coagulation using aluminum sulfate. *Desalination*, **225**, 301-311.

# Chapter 3

## A COAGULATION/FLOCCULATION-CENTRIFUGATION STEP TO REDUCE THE FOG CONTENT OF GDWW BEFORE USE AS SUBSTRATE IN AN UPFLOW ANAEROBIC SLUDGE BED REACTOR


### SUMMARY

Grain distillery wastewater (GDWW), characterised by a high concentration of fats, oils and grease (FOG), was treated by several commercially available coagulation/flocculation products in order to remove sufficient amounts of FOG and total soluble solids (TSS). Different coagulation/flocculation products were evaluated in combination with a centrifugation step for improved sedimentation and separation. The FOG removal remained between 90 and 97% for the ferric chloride ($FeCl_3$) and Ferrifloc 1820 treatments, respectively, whereas TSS removal ranged between 56 and 93%, respectively. The use of a high molecular weight polymer (Ultrafloc 5000) and an aluminium chlorohydrate (Ultrafloc 3800) proved to be less effective treating GDWW with FOG removal ranging from 72 to 86%.


### INTRODUCTION

The purification of wastewater from various industrial processes is becoming a problem worldwide, mainly due to the increasingly restricted amounts of water suitable for direct use, the high price of purification installations and the necessity of utilising waste products (Lalov *et al.*, 2000). Waste minimisation is an important aspect for any industry, as it not only reduces the consumption of potable water but also decreases the amount of wastewater generated (Melamane *et al.*, 2007). Due to the on-going development of various industry sectors such as the beverage industry, textile industry, electronic industry and food industry, large amounts of wastewater are produced during the processes (Kuang, 2002; Piya-Areetham *et al.*, 2006). Water is a key process medium in most industries and is mainly used for preparation, cleaning, sanitation, heating, cooling, floor washing (Willey, 2001; Nataraj *et al.*, 2006; Sarkar *et al.*, 2006). Among these types of wastewater, distillery wastewater is highly loaded with organic matter, which if discharged into water sources without treatment, may cause severe environmental pollution (Basu, 1975; Driessen *et al.*, 1994).

Whisky is prepared from fermented cereals which are further matured in oak barrels. The cereals used for whisky production include corn, rye, barley and wheat. The

production process involves malting, mashing, fermentation, distillation and maturation. All of these steps contribute to the composition of grain distillery wastewater (GDWW) (Goodwin & Stuart, 1994; Goodwin *et al.*, 2001; Uzal *et al.*, 2003; Csar, 2009). The wastewater produced is characterised by an extremely high chemical oxygen demand (COD) (Pescod, 1992) (10 000 – 60 000 $mg.L^{-1}$), high biochemical oxygen demand (BOD) (25 000 – 30 000 $mg.L^{-1}$), low pH (3.3 – 4.3), foul odour and a dark brown colour (Gao *et al.*, 2007; Satyawali & Balakrishnan, 2007; Sowmeyan & Swaminathan, 2008; Mohana *et al.*, 2009). Between 16 and 21 L of wastewater can be produced for each litre of grain whisky produced (Tokuda *et al.*, 1998).

The GDWW is rich in fats, oils and grease (FOG) which can be problematic when a biological system is used as a primary treatment option (Cammarota & Freire, 2006). The formation of a lipid coating around the biological flocs causes several problems within both aerobic and anaerobic treatment systems. In aerobic treatment systems the reduction of oxygen transfer can severely hamper the efficiency of the microorganisms leading to a loss in system performance (Cavaleiro *et al.*, 2001; Cammarota & Freire, 2006). The adsorbed lipid's specific gravity and the inability of the sludge to settle will lead to sludge bed washout in anaerobic treatment systems such as UASB reactors (Cavaleiro *et al.*, 2001; Chipasa & Mdrzycka, 2008).

Coagulation/flocculation treatment is one of the most significant physico-chemical steps to reduce soluble solids and colloidal material which may contribute to wastewater turbidity as well the reduction of COD and BOD content of the water (Al-Mutairi *et al.*, 2004; Sarkar *et al.*, 2006). Coagulation/flocculation is normally required to treat wastewater containing high amounts of small particles (<5 μm) and involves combining these particles (colloidal or suspended) and other organic material into larger aggregates, thereby facilitating the sedimentation or flotation of these flocs (Hogg, 2000; Zhou *et al.*, 2008). Various types of coagulation/flocculation products are in use today and can be classified into different groups namely: inorganic (aluminium sulphate, aluminium chloride, polyaluminium sulphate, polyaluminium chloride, ferric chloride, ferric sulphate, ferrous sulphate), synthetic polymers, microbial (extracellular biopolymeric flocculants) and natural occurring agents (chitosan, starches, tannins, alginates) (Salehizadeh & Shojaosadati, 2001; Dominguez *et al.*, 2005).

The process of coagulation involves the destabilisation of the anionically charged suspended colloidal materials (Chesters *et al.*, 2009). Destabilisation can be a result of charge neutralisation or the enmeshment within a metal hydroxide precipitate (Zhou *et al.*, 2008). Flocculation involves the bridging of particles by polymer chains, forming flocs or

larger aggregates. These flocs will either float or sink, making them easier to remove from the system (Chesters *et al.*, 2009). The effectiveness of this treatment will depend on the coagulation/flocculation agent used, dosage strength, pH and ionic strength of the solution, the concentration and nature of the organic compounds in the wastewater (Dominguez *et al.*, 2005; Zayas *et al.*, 2007). Particles within the solution carry a charge due to electrochemical interactions between the particles and surrounding solution and this charge is influenced by the solution's pH. Thus, pH control can greatly affect the coagulation efficiency of the flocculent (Hogg, 2000). Zayas *et al.* (2007) showed that increased pH can improve the efficiency when treating vinasse with a combined coagulation/flocculation-electrochemical oxidation treatment. It was found that COD removal increased from 54% (pH 4 - 6) to 84% (pH 6 - 8.4) using $FeCl_3$ (20 $g.L^{-1}$) as coagulant (Zayas *et al.*, 2007). Sarker *et al.* (2006) treated dairy wastewater with alum (aluminium sulphate) and ferric chloride and found that an increased pH (6.5 - 8.0) increased the effectiveness of dosing and a subsequent higher degree of flocculation and settling, achieved. Zhou *et al.* (2008) found that increased dosing at constant pH increased colour and COD reduction to 90 and 60 %, respectively when treating secondary yeast wastewater. Al-Mutairi *et al.* (2004) treated slaughterhouse effluent with a combination of an alum salt ($Al_2(SO_4)_3.H_2O$) and a commercially available polymer. The COD and soluble solids (SS) removal ranged from 3 - 20% and 98 - 99%, respectively using an alum salt (100 - 1000 $mg.L^{-1}$) at a pH of 4 - 9 (Al-Mutairi *et al.*, 2004). When using the polymer, Al-Mutairi and co-workers removed up to 43% COD and 96% SS.

The aim of this investigation was to use a coagulation/flocculation-centrifugation step to obtain FOG-reduced GDWW to be used in subsequent UASB treatment investigations (in Chapter 4 and 5, on the efficacy of the UASB process treating FOG-reduced GDWW). This was done by utilising a coagulation/flocculation-centrifugation step, making use of a small range of commercially available coagulation/flocculation products at prescribed dosages and uniform conditions. A centrifugation step was applied as a means to separate the formed aggregates from the FOG-reduced GDWW. The coagulation/flocculation-centrifugation combination achieving the best FOG removal was chosen to produce the FOG-reduced GDWW required for subsequent investigations.

## MATERIALS AND METHODS

### Wastewater

The GDWW was obtained from a distillery in Wellington, South Africa. The GDWW had a pH and COD ranging from 3.5 to 4.0 and 22 000 to 28 000 mg.$L^{-1}$, respectively. During the trial the GDWW was stored in 25 L containers at –18°C until required. The GDWW was then allowed to thaw and stored at 4°C.

### Pre-treatment

Based on their chemical structure and characteristics four different coagulation/flocculation products were evaluated (Table 3.1). These products included an inorganic chloride solution, a polymer and chloride solution, an aluminium chlorohydrate solution and an high molecular weight polyelectrolyte solution. The following compounds, based on their chemical structure, were received from Chlorchem (Kempton Park, South Africa): Ferric chloride ($FeCl_3$); Ferrifloc 1820; Ultrafloc (UF) 3800 and UF 5000. Each product was evaluated in combination with a centrifugation step, in terms of FOG and COD removal efficiency from GDWW. An additional three treatments were trialled consisting of a double centrifugation step with either $FeCl_3$ or Ferrifloc 1820 as well as a centrifugation step without the use of an addition of a coagulation/flocculation product.

Ferric chloride ($FeCl_3$) is an inorganic chloride solution which has a wide treatment range from sewage to industrial strength wastewater. The floc formation ability of $FeCl_3$ can be enhanced with a polyelectrolyte coagulant (Ultrafloc 5000) (Anon., 2009a). Ferrifloc 1820 is a combined cationic polymer and $FeCl_3$ solution which is mainly used for the clarification of a wide range of surface waters and industrial effluent. It is most effective when dosed into a turbulent system where maximum conditioning time is allowed (Anon., 2009b). Ultrafloc 3800 is an aluminium chlorohydrate and is characterised as superior coagulation agent for the clarification of raw water and effluent water (Anon., 2009c). Ultrafloc 5000 is a polyquaternary amine and is used for the clarification of surface waters (Anon., 2009d). For this study it was decided to use the maximum concentration allowed for drinking water in South Africa when determining the concentration of each agent used in the trial (Table 3.1) (Anon., 2009a; 2009b; 2009c; 2009d).

The pre-determined amount of coagulant/flocculant was added to 1.5 L of GDWW in a 2 L Schott bottle. The coagulant was mixed with the GDWW on a Labcon shaker for two min at 130 rpm. After mixing, 200 g of the GDWW was weighed off into each of six 250 mL centrifuge bottles and centrifuged (Beckman Coulter TJ-25) at 10 000 rpm for 10

min (15°C). During centrifugation three fractions were separated - a light fraction, a heavy (solids) fraction and the supernatant. Most of the FOG and TSS were entrapped in the heavy fraction. The heavy fraction was discarded whereas the light fraction and supernatant was refrigerated until analysis.

**Table 3.1** The different combination of coagulation/flocculation products used in the trial (Anon., 2009a; 2009b; 2009c; 2009d).

| Pre-treatment | Chemical structure | Concentration ($mg.L^{-1}$) |
|---|---|---|
| Ferric chloride ($FeCl_3$) → Centrifuge | Inorganic chloride solution | 250 |
| Ferrifloc 1820 (FF1820) → Centrifuge | Polymer and $FeCl_3$ solution | 100 |
| Centrifuge → $FeCl_3$ → Centrifuge | | 250 |
| Centrifuge → FF1820 → Centrifuge | | 100 |
| Ultrafloc 3800 → Centrifuge | Aluminium chlorohydrate (ACH) | 110 |
| Ultrafloc 5000 → Centrifuge | High molecular weight polyelectrolyte solution | 100 |
| Centrifuge (10 000 rpm) | | |

An additional treatment consisting of a double centrifugation step was also evaluated comparing ferric chloride and Ferrifloc 1820. After an initial centrifugation of raw GDWW, the supernatant was decanted and re-treated with the coagulant/flocculant agent before the onset of a secondary centrifugation. The heavy fraction from centrifugation was weighed and compared to for the different coagulation/flocculation treatments.

*Analytical methods*

The analytical parameters that were determined on the raw GDWW and treated GDWW included COD, FOG and TSS (APHA, 1998). The mass of the solid fraction was measured after centrifugation.

Determination of FOG was modified from the APHA (1998) method as follows: Wastewater samples (100 g) were acidified to pH 2 with hydrochloric acid (HCl) (2 M), weighed (50 g) and transferred to a separator funnel. Ethanol (absolute) (100 mL), 20 mL n-hexane and 20 mL diethyl ether (1:1) were added to the separator funnel, the sample was shaken vigorously and for the layers left to settle and separate. The bottom layer was drained and the top layer (FOG concentrate) was collected. The drained layer

was re-treated three more times with 20 mL n-hexane and diethyl ether (1:1) to extract more oils from the sample. The cumulative solvent sample was distilled in a rotavap (Büchi Rotavapor R-114) at 60°C and the distilled sample was measured gravimetrically and quantified to $mg.L^{-1}$ FOG.

## RESULTS AND DISCUSSION

The efficiency of each treatment in terms of FOG removal is shown in Fig. 3.1. In Fig. 3.2 the efficiency of each treatment with regards to FOG removal, TSS removal and centrifuge mass generated, is shown. Slight differences in the raw composition occurred, as can be seen in Fig. 3.1. These variations are due to the inherent variation during the production process.

### Ferric chloride

The FOG concentration of the raw GDWW batches measured was 1 300 and 1 700 $mg.L^{-1}$, respectively, during the $FeCl_3$ treatments. Remaining FOG in the treated GDWW ranged from 50 to 124 $mg.L^{-1}$, resulting in a FOG removal efficiency ranging from 91 to 97% (average *ca.* 94%) (Figs. 3.1 and 3.2). The remaining TSS ranged from 0.135 to 0.173 $g.L^{-1}$, resulting in a removal efficiency in the range of 74 to 93% (average *ca.* 86.7%) (Fig. 3.2). The Centrifuged mass (solids fraction) collected after treatment ranged from of 2 to 2.75 g for 200 g GDWW (Fig. 3.2).

Ferric chloride action is based on its ability to dissolve in an aqueous solution and then becoming hydrated or hydrolysed to form various manomeric and polymeric species. The low pH of GDWW (3.5 – 4.0) resulted in hydrolysis and the formation of ferric hydroxide, $Fe(OH)_3$. These metal hydroxide polymers have a large surface area, amorphous structure and are positively charged. Their hydrophobic nature causes them to absorb any anionic organic compounds and become insoluble (Al-Mutairi *et al.*, 2004; Dominguez *et al.*, 2005; Zayas *et al.*, 2007). An increase in the pH would improve the effectiveness of ferric chloride action (Al-Mutairi *et al.*, 2004; Dominguez *et al.*, 2005; Zayas *et al.*, 2007). However, it was decided not to adjust the pH of the GDWW in this study due to the cost saving implication when applied in a full scale operation. Compared to all the other treatments $FeCl_3$ was used at the highest possible concentration (250 $mg.L^{-1}$), as per the supplier's instruction, explaining the high efficiency in FOG and TSS removal.

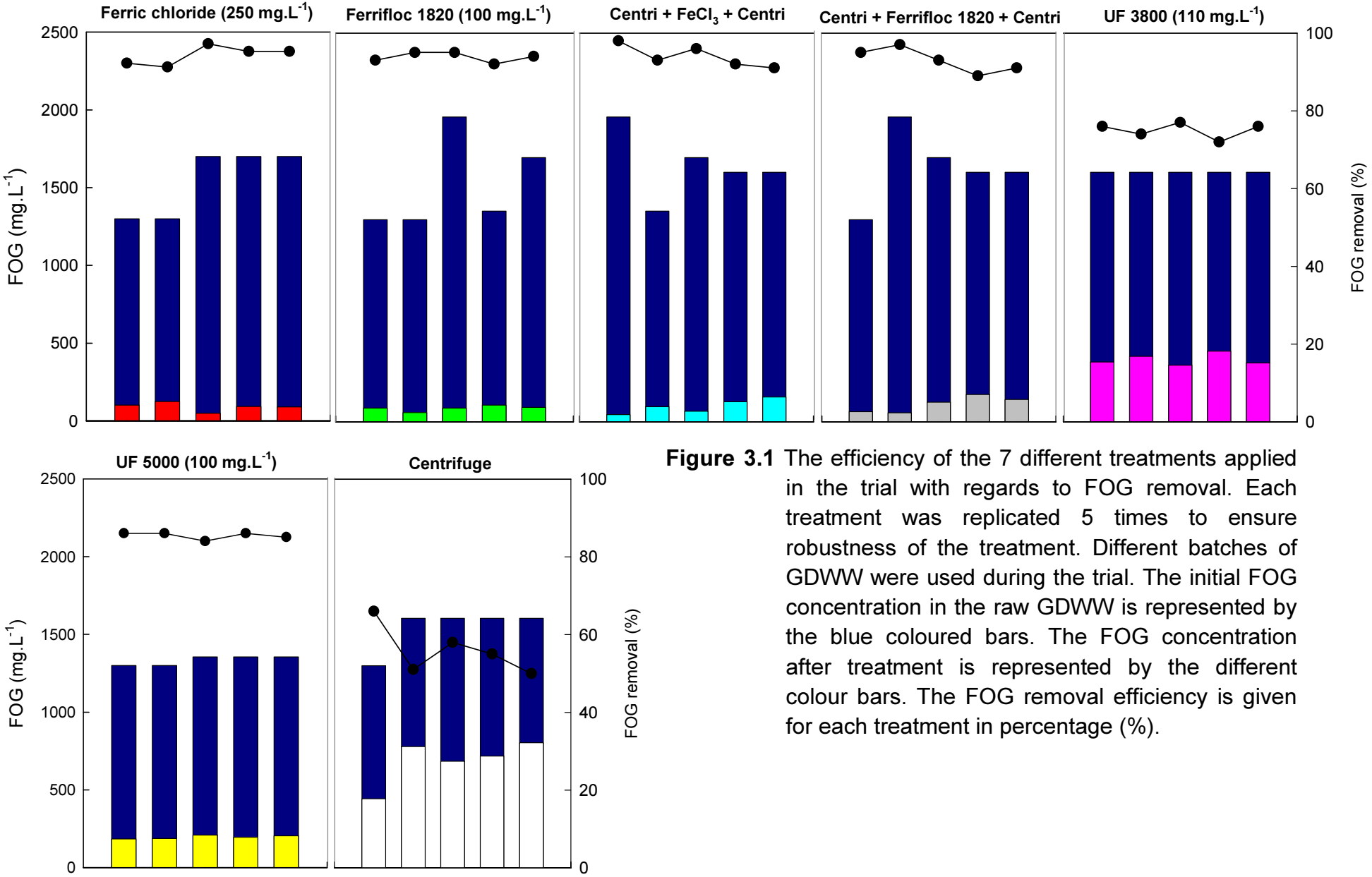


**Figure 3.1** The efficiency of the 7 different treatments applied in the trial with regards to FOG removal. Each treatment was replicated 5 times to ensure robustness of the treatment. Different batches of GDWW were used during the trial. The initial FOG concentration in the raw GDWW is represented by the blue coloured bars. The FOG concentration after treatment is represented by the different colour bars. The FOG removal efficiency is given for each treatment in percentage (%).

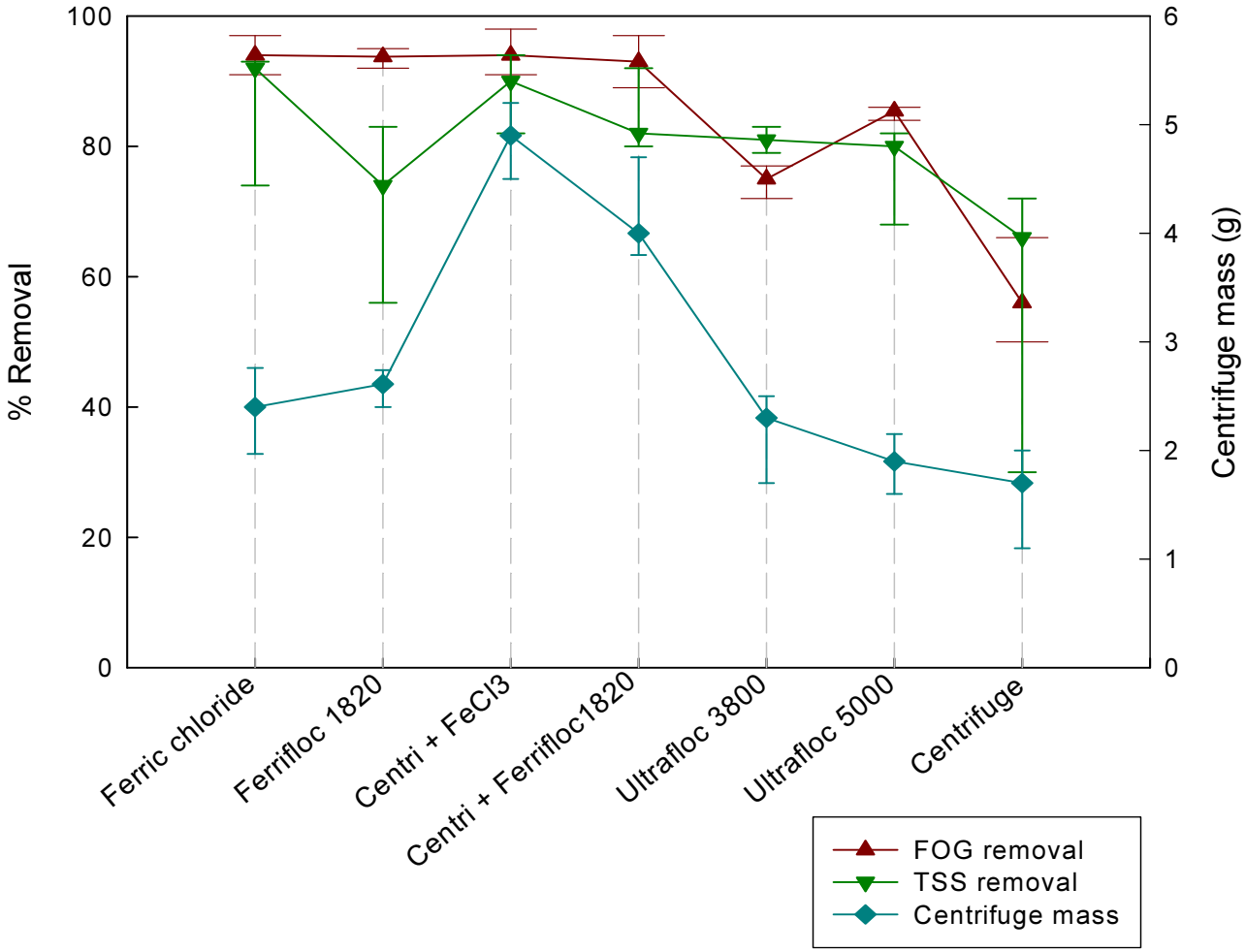


**Figure 3.2** The effectiveness of each treatment with regards to FOG removal, TSS removal and centrifuge mass removal. The bars represent the median of the minimum and maximum effectiveness of each treatment.

**Ferrifloc 1820**

The FOG concentration of the raw GDWW batches used ranged from 1 354 to 1 960 mg.L$^{-1}$ for the Ferrifloc 1820 treatments (Fig. 3.1). The amount of FOG remaining in the treated GDWW after flocculation ranged from 60 to 108 mg.L$^{-1}$, resulting in a removal efficiency from 92 to 95% (avg. 93.8%) (Figs. 3.1 and 3.2). Ferrifloc 1820 showed efficient FOG removal capacity comparable to ferric chloride, as well as at a lower concentration. The toxicological effects of Ferrifloc 1820 only permited the use of 100 mg.L$^{-1}$ compared to 250 mg.L$^{-1}$ for $FeCl_3$ (Anon., 2009a; 2009b).

The TSS removal was lower compared to $FeCl_3$ as the remaining TSS ranged from 0.25 to 0.398 g.L$^{-1}$. The TSS removal efficiency ranged from 56 to 83% (avg. 72%) (Fig. 3.2). This was the lowest efficiency of all the coagulation/flocculation agents. Ferrifloc

1820 is a combination of ferric chloride and a polyquaternary amine (Anon., 2009b). This high weight molecular polymer requires a more turbulent and longer mixing time. Using the same mixing technique, i.e. mixing the flocculant/coagulant with GDWW for 2 min followed by centrifugation, for all treatments could possibly explain the lower TSS removal experienced for this flocculant/coagulant. The centrifuged mass removal ranged between 2.4 and 2.74 g for 200 g GDWW (Fig. 3.2).

**Centrifuge + Ferric chloride**

It was initially proposed that a double centrifugation treatment would considerably improve FOG and TSS removal from GDWW. An initial centrifugation would remove a certain percentage of FOG and TSS and possibly enhance the effectiveness of the subsequent flocculation and centrifugation treatment. However, it was found that following a double centrifugation treatment did not improve the FOG and TSS removal capacity considerably over a single centrifugation treatment. It was only found that using a double centrifugation step helped maintain a stable removal efficiency.

The FOG concentration ranged from 1 354 to 1 960 $mg.L^{-1}$ (Fig. 3.1) for the raw GDWW. The remaining FOG concentration after the treatment ranged from 37 to 160 $mg.L^{-1}$ (Fig. 3.1 and 3.2). FOG removal efficiency thus ranged from 91 to 98% (avg. 94%) following a double centrifugation step compared to the range of 91 to 97% (avg. 94%) for a single centrifugation treatment with $FeCl_3$. The remaining TSS after the double centrifugation was lower than a single centrifugation treatment, ranging from 0.14 to 0.27 $g.L^{-1}$. The TSS removal efficiency ranged from 82 to 94% (avg. 89%) compared to the wider efficiency range of 74 to 93% (avg. 86%) for a single centrifugation treatment (Fig. 3.2). The double centrifugation step did, however, vastly improved the amount of solids fraction collected. The double centrifugation mass removed ranged from 4.5 to 5.2 g compared to the single centrifugation of between 2.0 and 2.75 g for 200 g GDWW (Fig. 3.2).

**Centrifuge + Ferrifloc 1820**

The double centrifugation step in combination with Ferrifloc 1820 did not result in an improved FOG removal efficiency compared to a single centrifugation technique. Raw GDWW FOG concentration ranged from 1 300 to 1960 $mg.L^{-1}$ during the treatments (Fig. 3.1). Following a double centrifugation step, it did yield a higher FOG removal efficiency for Ferrifloc 1820. However, a single centrifugation treatment resulted in a more stable removal efficiency observed during the test runs as the FOG removal ranged from

89 to 97% (avg. 93%) compared to the single centrifugation witched ranged from 92 to 95% (avg. 93.8%) (Figs. 3.1 and 3.2). The TSS, however, showed increased stability as well as increased removal efficiency following a double centrifugation treatment during the treatment runs as the remaining TSS ranged from 0.16 to 0.39 $g.L^{-1}$. The TSS removal ranged from 80 to 92% (avg. 84.7%) compared to the single centrifugation treatment ranging from 56 to 83% (avg. 72%) (Fig. 3.2). The centrifuged mass removed ranged from 3.80 to 4.70 g for 200 g GDWW, improving over the single centrifugation treatment ranging from 2.40 to 2.74 g (Fig. 3.2).

**Ultrafloc 3800**

The FOG concentration of the raw GDWW batch used was 1 605 $mg.L^{-1}$ during all Ultrafloc 3800 treatments (Fig. 3.1). The FOG concentration of the GDWW after flocculation was between 380 and 454 $mg.L^{-1}$, resulting in a removal efficiency of between 72 and 77% (avg. 75%) (Figs. 3.1 and 3.2). The removal efficiency of this treatment was the lowest of all the coagulation/flocculation agents. The remaining TSS ranged from 0.33 to 0.41 $g.L^{-1}$, resulting in a removal efficiency ranging from 79 to 83% (avg. 81%) (Fig. 3.2). The centrifuged mass removed ranged from 1.70 to 2.30 g per 200 g GDWW (Fig. 3.2).

The lower removal efficiency might be attributed to Ultrafloc 3800's treatment characteristics. Ultrafloc 3800 is an aluminium chlorohydrate, a form of a polyaluminium chloride. Aluminium chloride efficiency is based on the pH of the wastewater, the low pH of GDWW may have altered the removal efficiency of this compound (Gabelich *et al.*, 2006; Wang *et al.*, 2008).

**Ultrafloc 5000**

The FOG concentration of the raw GDWW ranged between 1 300 and 1 354 $mg.L^{-1}$ during the treatments (Fig. 3.1). Ultrafloc 5000 proved to be more effective than UF 3800 although less effective than $FeCl_3$ and Ferrifloc 1820 with regards to FOG removal. The FOG concentration of the GDWW after flocculation was between 185 and 210 $mg.L^{-1}$, resulting in a relatively stable removal efficiency ranging from 84 to 86% (avg. 85.4%) (Figs. 3.1 and 3.2). The remaining TSS ranged from 0.25 to 0.30 $g.L^{-1}$, resulting in a removal efficiency ranging from 68 to 82 % (avg. 76%) whereas the centrifuged mass removed from 1.6 to 2.15 g per 200 g GDWW (Fig. 3.2).

The UF 5000 is a high molecular weight polyelectrolyte and when these coiled chain polymers dissolve in water the charged areas on the chain start to repel each other,

resulting in an uncoiled structure which increases the viscosity of the solution (Anon., 2009d; Chesters *et al.*, 2009). This reaction is time consuming and due to the treatment technique followed (relatively short mixing time and mixing not vigorously enough) for the different combinations the UF 5000 could have performed ineffectively (Chesters *et al.*, 2009). Increasing the reaction time needed and the turbulence of the wastewater may result in improved UF 5000 performance.

**Centrifugation**

It was expected that a single centrifugation would perform the poorest with regards to FOG and TSS removal from GDWW. The FOG concentration of the raw GDWW ranged from 1 300 to 1 605 $mg.L^{-1}$ during these treatments (Fig. 3.1). The FOG concentration after centrifugation was in the range of 445 to 805 $mg.L^{-1}$. This is a removal efficiency in the range of 50 to 66% (avg. 56%), which was well below any of the other treatments followed (Figs. 3.1 and 3.2). The remaining TSS ranged from 0.55 to 0.65 $mg.L^{-1}$, resulting in a efficiency of 30 to 72% (avg. 61%), also well below any of the other treatments (Fig. 3.2). Centrifuged mass removed ranged from 1.1 to 2.0 g for 200 g GDWW (Fig. 3.2).

## CONCLUSION

The aim of this investigation was to develop a coagulation/flocculation-centrifugation step to obtain FOG-reduced GDWW to be used in subsequent UASB reactor treatment investigations. Using different commercially available coagulation/flocculation products, $FeCl_3$-containing coagulation/flocculation products proved to be the most effective at FOG removal. Ferrifloc 1820 (polymer and $FeCl_3$ solution) and ferric chloride removed in excess of 90% FOG from GDWW during all treatment replications.

This technique will serve as pre-treatment for the following UASB reactor research chapters. Based on the data obtained it was decided to use $FeCl_3$ in combination with a single centrifugation as pre-treatment. However, a build-up of the metal can occur in the sludge generated during the pre-treatment. This can be undesirable if the final sludge is to be used as animal feed because of the pro-oxidant and toxic effects at high concentrations (Xu *et al.*, 2001). Disposal or reuse thereof will require further investigation.

A better understanding of the composition of the GDWW is required to identify even more suitable and effective coagulants/flocculants. Furthermore, this investigation only focused on reducing FOG and solids by evaluating different commercially coagulation/flocculation products and negating several important efficiency parameters

such as different dosages, mixing retention times, various pH conditions and methods of separation (such as dissolved air floatation). To fully maximise the efficiency of the coagulant/flocculant used all the above mentioned parameters must be investigated. By improving the FOG and solids removal efficiency it can result in a more effective secondary treatment. It is also important to take into consideration that the large scale implementation of a continuous coagulation/flocculation-centrifugation pre-treatment system will certainly have different removal efficiencies than a laboratory-scale batch system and this needs to be examined.

## REFERENCES

Al-Mutairi, N.Z., Hamoda, M.F. & Al-Ghusain, I. (2004). Coagulant selection and sludge conditioning in a slaughterhouse wastewater treatment plant. *Bioresource Technology*, **95**, 115-119.

Anon. (2009a). NCP Chlorchem. [WWW document] Ferric chloride. [www.ncp.co.za]. 11/12/09.

Anon. (2009b). NCP Chlorchem. [WWW document] Ferrifloc 1820. [www.ncp.co.za]. 11/12/09.

Anon. (2009c). NCP Chlorchem. [WWW document] U3000 ACH Blends. [www.ncp.co.za]. 11/12/09.

Anon. (2009d). NCP Chlorchem. [WWW document] Polyamines. [www.ncp.co.za]. 11/12/09.

APHA (1998). *Standard methods for the examination of water and wastewater*, 20th ed. Pp. 2:26-28, 22:54-58, 24:86-87, 24:149-150, 145:113-117, 145:134-139. Washington D. C.

Basu, A.K. (1975). Characteristics of distillery wastewater. *Journal (Water Pollution Control Federation)*, **47**, 2184-2190.

Cammarota, M.C. & Freire, D.M.G. (2006). A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresource Technology*, **97**, 2195-2210.

Cavaleiro, A.J., Alves, M.M. & Mota, M. (2001). Microbial and operational response of an anaerobic fixed bed digester to oleic acid overloads. *Process Biochemistry*, **37**, 387-394.

Chesters, S., Darton, E., Gallego, S. & Vigo, F. (2009). The safe use of cationic flocculants with reverse osmosis membranes. *Desalination and Water Treatment*, **6**, 144-151.

Chipasa, K.B. & Mdrzycka, K. (2008). Characterization of the fate of lipids in activated sludge. *Journal of Environmental Sciences*, **20**, 536-542.

Csar, P. (2009). Pure Single Malt Scotch Whisky. [WWW document] The Making of Whisky. [www.editme.co.za]. 04/03/09.

Dominguez, J., de Heredia, J., Gonzalez, T. & Sanchez-Lavado, F. (2005). Evaluation of ferric chloride as a coagulant for cork processing wastewaters. Influence of the operating conditions on the removal of organic matter and settleability parameters. *Industrial and Engineering Chemistry Research*, **44**, 6539-6548.

Driessen, W.J.B.M., Tielbaard, M.H. & Vereijken, T.L.F.M. (1994). Experience on anaerobic treatment of distillery effluent with the UASB process. *Water science and technology*, **30**, 193 - 209.

Gabelich, C.J., Ishida, K.P., Gerringer, F.W., Evangelista, R., Kalyan, M. & Suffet, I.H.M. (2006). Control of residual aluminum from conventional treatment to improve reverse osmosis performance. *Desalination*, **190**, 147-160.

Gao, M., She, Z. & Jin, C. (2007). Performance evaluation of a mesophilic (37 °C) upflow anaerobic sludge blanket reactor in treating distiller's grains wastewater. *Journal of Hazardous Materials*, **141**, 808-813.

Goodwin, J.S., Finlayson, J.M. & Low, E.W. (2001). A further study of the anaerobic biotreatment of malt whisky distillery pot ale using an UASB system. *Bioresource Technology*, **78**, 155-160.

Goodwin, J.S. & Stuart, J.B. (1994). Anaerobic digestion of malt whisky distillery pot ale using upflow anaerobic sludge blanket reactors. *Bioresource Technology*, **49**, 75-81.

Hogg, R. (2000). Flocculation and dewatering. *International Journal of Mineral Processing*, **58**, 223-236.

Kuang, Y. (2002). Enhancing Anaerobic Degradation of Lipids in Wastewater by Addition of Co-substrate. In: PhD in School of Environmental Science, Murdoch University.

Lalov, I.G., Guerginov, I.I., Krysteva, M.A. & Fartsov, K. (2000). Treatment of waste water from distilleries with chitosan. *Water Research*, **34**, 1503-1506.

Melamane, X., Strong, P. & Burgess, J. (2007). Treatment of Wine Distillery Wastewater: A Review with Emphasis on Anaerobic Membrane Reactors. *S. Afr. J. Enol. Vitic*, **28**, 25.

Mohana, S., Acharya, B.K. & Madamwar, D. (2009). Distillery spent wash: Treatment technologies and potential applications. *Journal of Hazardous Materials*, **163**, 12-25.

Nataraj, S.K., Hosamani, K.M. & Aminabhavi, T.M. (2006). Distillery wastewater treatment by the membrane-based nanofiltration and reverse osmosis processes. *Water Research*, **40**, 2349-2356.

Pescod, M. (1992). Wastewater treatment and use in agriculture-FAO irrigation and drainage paper 47. *Food and Agriculture Organization of the United Nations*.

Piya-Areetham, P., Shenchunthichai, K. & Hunsom, M. (2006). Application of electrooxidation process for treating concentrated wastewater from distillery industry with a voluminous electrode. *Water Research*, **40**, 2857-2864.

Salehizadeh, H. & Shojaosadati, S.A. (2001). Extracellular biopolymeric flocculants: Recent trends and biotechnological importance. *Biotechnology Advances*, **19**, 371-385.

Sarkar, B., Chakrabarti, P., Vijaykumar, A. & Kale, V. (2006). Wastewater treatment in dairy industries—possibility of reuse. *Desalination*, **195**, 141-152.

Satyawali, Y. & Balakrishnan, M. (2007). Removal of color from biomethanated distillery spentwash by treatment with activated carbons. *Bioresource Technology*, **98**, 2629-2635.

Sowmeyan, R. & Swaminathan, G. (2008). Effluent treatment process in molasses-based distillery industries: A review. *Journal of Hazardous Materials*, **152**, 453-462.

Tokuda, M., Ohta, N., Morimura, S. & Kida, K. (1998). Methane fermentation of pot ale from a whisky distillery after enzymatic or microbial treatment. *Journal of Fermentation and Bioengineering*, **85**, 495-501.

Uzal, N., Gökçay, C.F. & Demirer, G.N. (2003). Sequential (anaerobic/aerobic) biological treatment of malt whisky wastewater. *Process Biochemistry*, **39**, 279-286.

Wang, J., Guan, J., Santiwong, S.R. & Waite, T.D. (2008). Characterization of floc size and structure under different monomer and polymer coagulants on microfiltration membrane fouling. *Journal of Membrane Science*, **321**, 132-138.

Willey, R. (2001). Fats, oils, and greases: The minimization and treatment of wastewaters generated from oil refining and margarine production. *Ecotoxicology and Environmental Safety*, **50**, 127-133.

Xu, L., Sheldon, B., Carawan, R., Larick, D. & Chao, A. (2001). Recovery and characterization of by-products from egg processing plant wastewater using coagulants. *Poultry Science*, **80**, 57.

Zayas, T., Rómero, V., Salgado, L., Meraz, M. & Morales, U. (2007). Applicability of coagulation/flocculation and electrochemical processes to the purification of biologically treated vinasse effluent. *Separation and Purification Technology*, **57**, 270-276.

Zhou, Y., Liang, Z. & Wang, Y. (2008). Decolorization and COD removal of secondary yeast wastewater effluents by coagulation using aluminum sulfate. *Desalination*, **225**, 301-311.

# CHAPTER 4

# MONITORING THE EFFICIENCY OF AN UPFLOW ANAEROBIC SLUDGE BLANKET REACTOR TREATING FOG-REDUCED GRAIN DISTILLERY WASTEWATER


## SUMMARY

FOG-reduced Grain distillery wastewater (GDWW) was treated in a lab-scale upflow anaerobic sludge blanket (UASB) reactor (2 L) over a period of 331 days. FOG-reduced GDWW was obtained following coagulation/flocculation-centrifugation pre-treatment step developed. During the operational period different feeding parameters were attained to establish the ability of the UASB reactor to efficiently treat FOG-reduced GDWW. The COD removal increased from 60 to 85 % at an OLR of *ca.* 5.5 kgCOD.$m^{-3}$.$d^{-1}$ (pH = 7.50) while FOG removal remained between 45 and 70 %. The COD removal increased to 90 % after the attainment of an OLR *ca.* 10 kgCOD.$m^{-3}$.$d^{-1}$ (pH = 7.50) whereas FOG removal remained in the region of 55 and 65 %. The lowering of the substrate pH to 6.50 (from 7.50) at an OLR *ca.* 10kg COD.$m^{-3}$.$d^{-1}$ proved vital for this study. COD and FOG removal remained above 85 % and 50 %, respectively. Granule activity tests performed by the end of the trial showed UASB reactor granules exposed to FOG-reduced GDWW over a period of 331 days to have higher activity compared to seed granules in terms of methane production rate and cumulative methane production.


## INTRODUCTION

Grain distillery wastewater (GDWW) is the waste product of grain whisky distillation and may be classified as a high strength wastewater due to its unique set of characteristics. Characteristics such as having a high chemical oxygen demand (COD) value of up to 60 g.$L^{-1}$, total soluble solid content up to 10 g.$L^{-1}$, fats oils and grease (FOG) concentration up to 2 g.$L^{-1}$ and a low pH (3.5 – 4) gives GDWW a low biodegradability coefficient (Goodwin & Stuart, 1994; Gao *et al.*, 2007). If not treated correctly GDWW may have a severe environmental impact on land and water (Mendes & Castro, 2005; Cammarota & Freire, 2006). Governments worldwide, including South Africa, are setting more strict requirements for pollution control and over the past 20 years there has been a demand for more effective and novel treatment technologies (Lu *et al.*, 1995; Akunna & Clark, 2000; Walsdorff *et al.*, 2005).

Due to increasing costs involved with aerobic treatment options the on-going development of high rate bioreactors has led to anaerobic biological treatment systems

being adopted worldwide for the treatment of various types of wastewaters including distillery wastewater (Deepak, 1998). The development of these high rate systems has led to improved solids retention time (SRT) and shorter hydraulic retention times (HRT), thus enabling the treatment of high strength distillery wastewater (Chernicharo, 2007). The UASB reactor has become a popular and versatile anaerobic treatment system throughout the world, the system presents an attractive solution because of low operational cost, low energy consumption, compact design, low sludge production and production of methane ($CH_4$) as a potential energy source (Lettinga *et al.*, 1980; Forday & Greenfield, 1983; Goodwin *et al.*, 1990; Chernicharo, 2007). The UASB reactor operates as a suspended growth system (without the use of any packing material) with the active biomass being held in suspension by hydraulic design (Deepak, 1998; Tiwari *et al.*, 2006). Goodwin (1994) was able to successfully treat GDWW at a loading rate of 15 $kgCOD.m^{-3}.d^{-1}$. Uzal *et al.* (2003) used a two-stage UASB system to reduce up to 93% COD from distillery wastewater and further increased the COD reduction up to 99% during a subsequent aerobic treatment. Gao *et al.* (2007) successfully treated GDWW and achieved up to 97.3% COD reduction at an OLR between 5 and 48 $kgCOD.m^{-3}.d^{-1}$ with a HRT of 82 to 11 h.

Although the anaerobic treatment of this type of wastewater has been well documented, operational problems may still occur. The high FOG content of GDWW can present a treatment complexity to anaerobic digestion and UASB reactors. The two main problems associated with lipids during treatment include the adsorption of a lipid layer around the granules which can lead to sludge bed flotation/washout and the acute toxicity of long chain fatty acids (LCFA), an intermediate during lipid metabolism, on methanogens and acetogens during anaerobic digestion (Koster & Cramer, 1987; Alves *et al.*, 2001; Cavaleiro *et al.*, 2001; Pereira *et al.*, 2002). This may severely hinder the effectiveness of an UASB reactor to treat FOG-rich GDWW and an efficient pre-treatment is required in order to reduce the excess FOG in this type of wastewater.

A coagulation/flocculation treatment is one of the most important physico-chemical steps to reduce soluble solids and colloidal material which may contribute to wastewater turbidity as well as the reduction of COD and biochemical oxygen demand (BOD) content of the water (Sarkar *et al.*, 2006). The treatment involves the combining of particles (colloidal or suspended) and other organic material into larger aggregates, thereby facilitating the sedimentation or flotation of the flocs (Hogg, 2000; Zhou *et al.*, 2008). After successful flocculation the settled mass can be dewatered and the treated wastewater may undergo a secondary treatment.

The aim of this investigation is three-fold. The first objective is to achieve lab-scale UASB reactor start-up and maintain an organic loading rate similar to that required by a full-scale UASB at a local grain distillery. The second objective is to determine whether the lab-scale reactor efficiency can be maintained at a higher organic loading rate and reduced influent pH in terms of COD and FOG removal. The third objective is to determine the level of acclimatisation of the lab-scale UASB biomass by performing a granule activity test and comparing the activity to the initial granule activity.

## MATERIALS AND METHODS

### UASB reactor design

The UASB reactor (Fig. 4.1) was set up as described by McLachlan (2004) and Gie (2007). The lab-scale UASB reactor had a height of 1 m, diameter of 50 mm and an operational volume of 2 L. The substrate was semi-continuously fed from the bottom of the reactor using a peristaltic pump (Watson-Marlow SciQ 323) controlled by an electronic timer at an HRT of 24 h. Biogas formed during digestion exited the top of the column and was measured using a biogas meter, consisting of a manometric unit with an electronically controlled counter. The overflow of the UASB reactor drained through a U tube, preventing atmospheric oxygen entering the system, into a 2 L Schott bottle. Re-circulation, with an up-flow velocity set at 0.74 $m.h^{-1}$, was achieved by means of a second peristaltic pump (Watson-Marlow SciQ 323). The temperature of the UASB was set and maintained at 35ºC with an electronic controlled solid-state thermostat and heating tape and insulation (Meyer *et al.*, 1983).

### Reactor start-up and operation

Granular sludge was obtained from a full-scale UASB reactor treating winery distillery wastewater (WDWW) in Wellington, South Africa. This sludge was used to seed the laboratory scale UASB reactor. Start-up was initiated by feeding the UASB reactor with water containing 500 $mg.L^{-1}$ urea ($(NH_2)_2CO$) and 500 $mg.L^{-1}$ di-potassium hydrogen orthophosphate ($K_2HPO_4$) over a period of 24 h. Hereafter, the UASB reactor was fed with GDWW diluted with reactor effluent (initial COD *ca.* 500 $mg.L^{-1}$). After a successful start-up the substrate COD was step-wise increased until the UASB reactor could effectively treat wastewater with a COD of *ca.* 10 000 $mg.L^{-1}$. During the period of operation 1 mL of a trace element solution, a solution consisting of various micro nutrients specifically

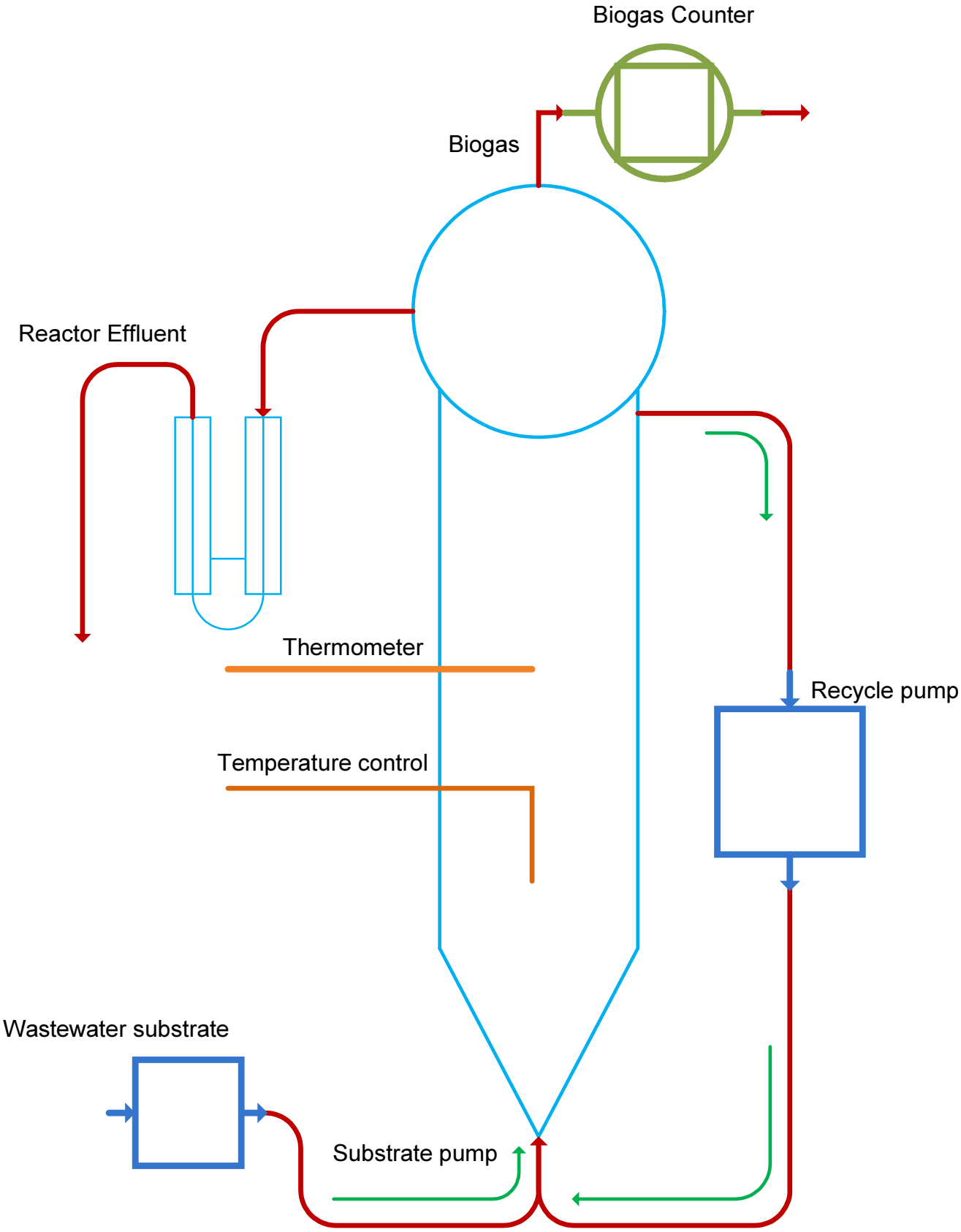


**Figure 4.1** Diagram of the setup of a laboratory-scale UASB reactor (McLachlan, 2004; Gie, 2007).

for anaerobic microorganisms was added to the UASB reactor on a weekly basis (Guida *et al.*, 2007). Additional urea and di-potassium orthophosphate were also added weekly to ensure a C:N:P (1000:7:1) ratio of the substrate.

**Wastewater**

The GDWW with a COD of 22 000 – 28 000 $mg.L^{-1}$ and pH of 3.40 – 3.70 was collected from a distillery in Wellington, South Africa (February to June of 2008 and 2009). The GDWW was stored in 25 L drums at -18ºC until required. Once required, a 25 L drum was allowed to thaw and was stored at 4ºC while in use. The GDWW underwent an initial pre-treatment in order to remove sufficient amounts of FOG and soluble solids (SS). After the pre-treatment the GDWW was diluted with reactor effluent to the specific COD concentration. The substrate pH was adjusted with 2 M potassium hydroxide (KOH) to between 6.50 and 7.50 depending on the strategic feeding approach of the UASB reactor during the trial.

**Pre-treatment of wastewater**

The pre-treatment of the GDWW was accomplished using a combination of a flocculation/coagulation and centrifugation, as developed during Chapter 3. The GDWW was initially dosed with $FeCl_3$ (Chlorochem, Kempton Park) to achieve the intended flocculation/coagulation followed by a centrifugation step to separate the light, supernatant and the heavy (solids) fractions.

To achieve flocculation/coagulation, 250 $mg.L^{-1}$ $FeCl_3$ was added to the GDWW and mixed at 130 rpm for 2 min in a Labcon shaker. The flocculated GDWW (200 g) was weighed off into 250 mL centrifuge bottles and centrifuged (Beckman Coulter TJ-25) at 10 000 rpm for 10 min at 15°C. The light fraction (FOG reduced) and supernatant were collected from the centrifuged sample whereas the heavy (FOG rich) fraction was discarded. The collected sample was stored at 4ºC until required as substrate for the UASB reactor. This method of storage was practiced throughout the study, including chapter 5.

**Analytical methods**

The parameters monitored on the GDWW and UASB effluent included pH, alkalinity (as $mg.L^{-1}$ $CaCO_3$), total suspended solids (TSS), ortho-phosphate ($PO_4^{3-}$), COD and FOG (APHA, 1998).

The FOG determination was modified as follows: Wastewater samples (100 g) were acidified to pH 2 using 2M hydrochloric acid (HCl). The samples were transferred into a separator funnel where 20 mL of n-hexane and diethyl ether (1:1) and 100 mL absolute ethanol (96 %) were added to the sample. The mixture was shaken vigorously and left to separate. The bottom layer (wastewater and ethanol) was drained and the upper layer (FOG concentrate) collected. The drained sample was remixed with n-hexane and diethyl ether (1:1) to extract more oils. This step was repeated two more times. The cumulative solvent was distilled in a rotavap (Bűchi Rotavapor R-114) at 60°C and the dry matter measured gravimetrically and quantified as $mg.L^{-1}$ FOG.

Biogas composition was determined by injecting a 0.2 mL biogas sample into a gas chromatograph (GC) (Varian 3300) (Sigge & Britz, 2007). The GC was equipped with a thermal conductivity detector and a 2.0 m x 3.0 mm i.d column filled with Hayesep Q (Supelco, Bellefonte, PA), 80/100 mesh. Helium was the carrier gas at a flow rate of 30 $mL.min^{-1}$ and the oven temperature was set at 55°C.

In order to determine the volatile fatty acid (VFA) concentrations, samples were prepared by mixing 3 mL wastewater sample, 1 mL 35 % formic acid and 2 µL n-hexanol (internal standard). A standard solution (acetic, propionic, iso-butyric, butyric, iso-valeric and valeric acid) was prepared in a 1 L volumetric flask by diluting 1 mL of each VFA and 0.5 mL n-hexanol in one part 35 % formic acid and 3 parts distilled water. Prepared samples (1 µL) were injected into a GC (Varian Model 3700), which was equipped with a flame ionisation detector and a 30 m bonded phase Nukol (Supelco, Inc., Belafonte, PA) fused silica capillary column with a diameter of 0.53 mm and a film thickness of 0.5 µm. Nitrogen was the carrier gas used at a flow rate of 6.1 $mL.min^{-1}$. The temperatures of the inlet and detector were set at 130°C and 300°C, respectively. For the first two minutes the column temperature was set at 105°C and then increased to 190°C at a rate of $10°C.min^{-1}$, and held for 10 min. VFA's were quantified, using Borwin ver. 1.2 integration software (JMBS Developments, Le Fontanil, France) (Sigge & Britz, 2007).

**Granule activity test**

Activity tests were performed on the UASB granules at day 0 of the trial and on day 331 (trial end) (O' Kennedy, 2000). An activity test was performed on the seed granules (day 0) of the UASB reactor to determine the initial granule activity. A second activity test was performed at the end of the trial to determine whether the granules showed increased activity after treating GDWW. Cumulative biogas (mL) and methane ($CH_4$) production rates were measured to determine the granule activity. Different test media were used, each

promoting the activity of certain microbial groups within the granule. The basic test media (BTM) was used as a control, where no microbial group within the granule are favoured, resulting in a measurement of the overall granule activity (Tables 4.1 and 4.2). The glucose test media (GTM) was used to determine the activity of the acidogens and the active methanogens while the acetic test media (ATM) was used to determine the activity of the acetoclastic methanogens. A fourth test media, consisting only of diluted FOG-reduced GDWW, was added to the activity test trial. The GDWW as test medium would determine the increase in granule activity as a result of the exposure to the same mixture over 331 days in the UASB reactor. The increased activity after 24 h incubation in GDWW could possibly be a verification of the proposed acclimatisation of granules as well as a possible microbial population shift.

During the initial activity test (day 0), 50 g of the sample granules were incubated in a 250 mL Schott bottle at 35°C for 48 h in 150 mL activation media (Table 4.3). This activation media was decanted after 24 h and replaced with fresh activation media. After 48 h of incubation was completed, duplicate granule samples (3 g) were placed in 20 mL glass vials for each specific test media (BTM, GTM, ATM and FOG-reduced GDWW). Each glass vial received 13 mL of the specific test media leaving a 6 mL headspace. The vials were sealed with butyl septa, enclosed with an aluminium cap and incubated at 35°C for 24 h. After 5, 10 and 24 h incubation the biogas volume was recorded by using a free moving 10 mL syringe with a 12 gauge needle. Biogas composition was gas chromatographically determined. On day 331, granules from the UASB reactor was sampled and tested. In this case, however, the already active granules did not undergo a pre-activation step.

**Table 4.1** Composition of basic test media (BTM) (O' Kennedy, 2000).

| Compound | Concentration ($g.L^{-1}$) |
|---|---|
| Glucose | 2.0 |
| Di- potassium hydrogen orthophosphate ($K_2HPO_4$) | 1.0 |
| Potassium di-hydrogen orthophosphate ($KH_2PO_4$) | 2.6 |
| Urea | 1.1 |
| Ammonium chloride ($NH_4Cl$) | 1.0 |
| Sodium sulphide ($Na_2S \cdot 9H_2O$) | 0.1 |
| Magnesium Chloride ($MgCl_2 \cdot 6H_2O$) | 0.1 |
| Yeast extract | 0.2 |
| pH | 7.1 |

**Table 4.2** Composition of the different test media used to determine the activity of specific microbial groups (O' Kennedy, 2000).

| Test media | Microbial group |
|---|---|
| Basic test media (BTM) | Control |
| Glucose test media (BTM + 2.0 $g.L^{-1}$ glucose) (GTM) | Acidogens |
| Acetic test media (BTM + 1.0 $g.L^{-1}$ acetic acid) (ATM) | Acetoclastic methanogens |
| FOG-reduced GDWW (*ca.* 5 000 $mg.L^{-1}$ COD) | Control |

**Table 4.3** Composition of the activation media used during the activity tests (O' Kennedy, 2000).

| Compound | Concentration ($g.L^{-1}$) |
|---|---|
| Glucose | 1.0 |
| Di- potassium hydrogen orthophosphate ($K_2HPO_4$) | 0.5 |
| Urea | 0.5 |

## RESULTS AND DISCUSSION

### Operational efficiency of UASB reactor treating GDWW

*UASB reactor*

In this research chapter, various UASB reactor substrate and effluent parameters were monitored. Substrate COD, effluent COD and COD reduction are shown in Fig. 4.2. The substrate pH, effluent pH, alkalinity and VFA measurements are shown in Fig. 4.3, whereas substrate FOG, effluent FOG and FOG reduction are shown in Fig. 4.4. The trial continued for 331 days during which the UASB reactor was exposed to different feeding strategies and operational conditions. The trial can be summarised into four phases (A to D), during each phase a different feeding strategy was followed with the aim of evaluating the reactor efficiency.

*Phase A (day 1 – day 130)*

The aim of phase A was to successfully achieve reactor start-up and maintain a loading rate of *ca.* 5 500 $mg.L^{-1}$ COD at a pH of 7.00. Throughout the trial substrate feed consisted

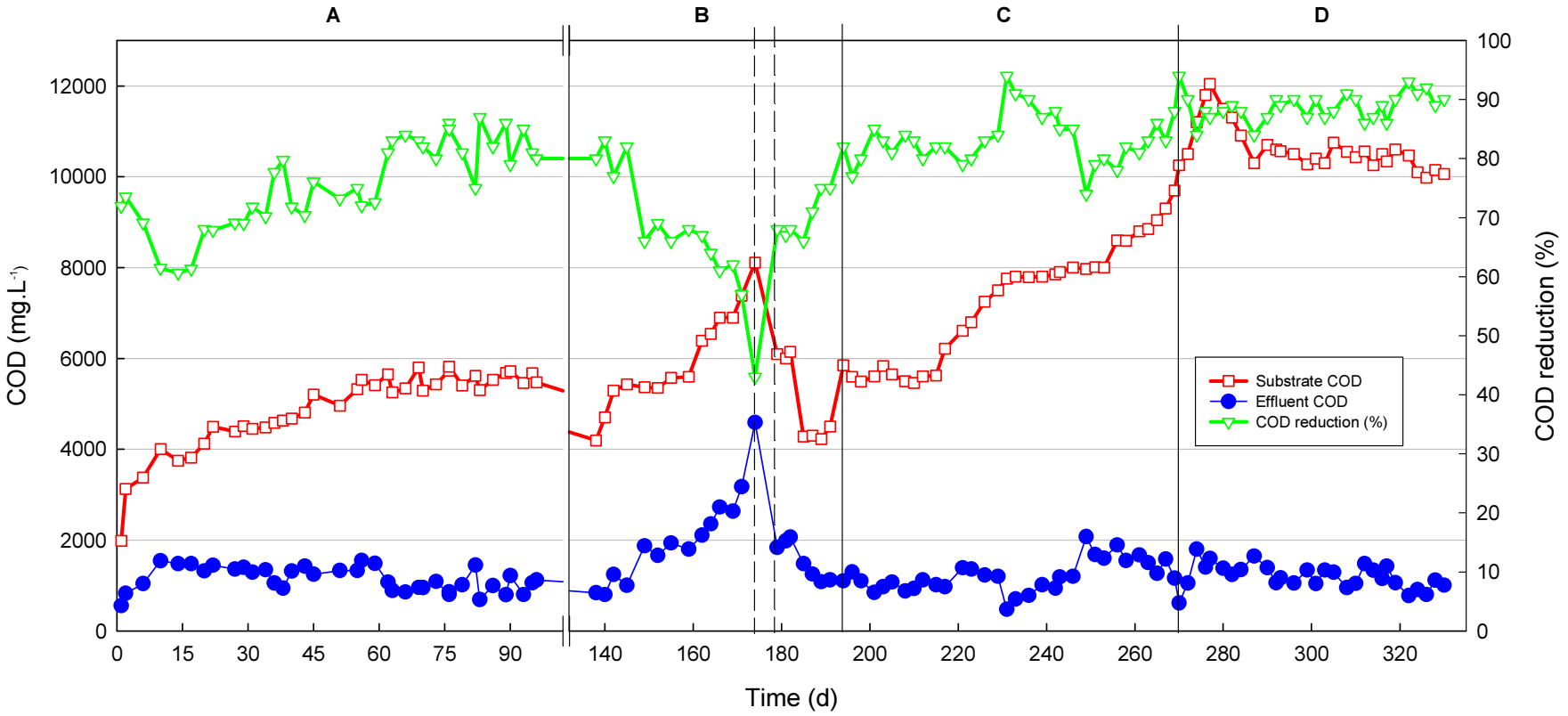


**Figure 4.2** Substrate COD, effluent COD and % COD reduction of the UASB reactor treating a FOG-reduced GDWW. Each phase is represented from A-D.

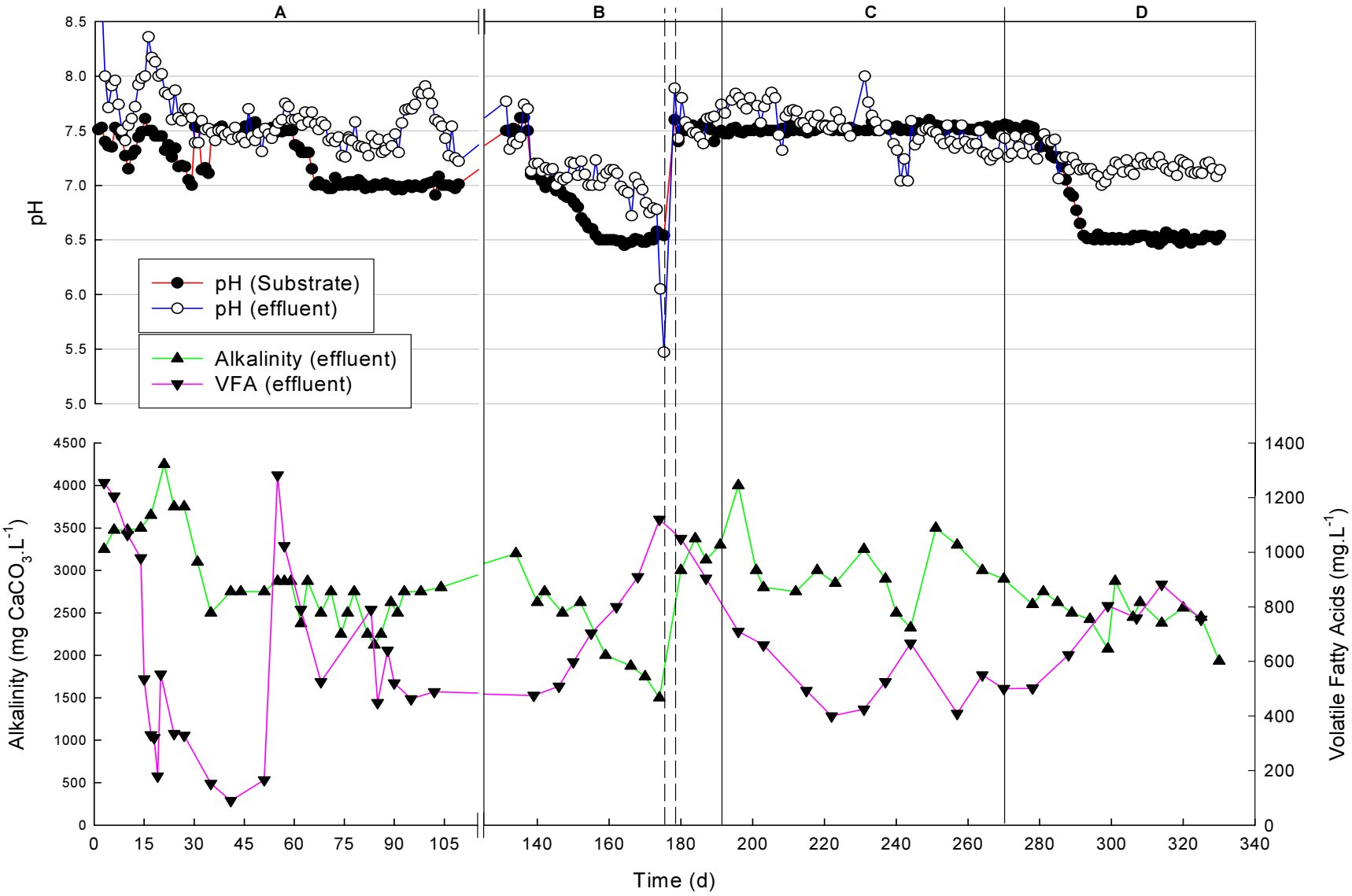


**Figure 4.3** Substrate pH, effluent pH, alkalinity and VFA levels in the UASB reactor treating FOG-reduced GDWW.

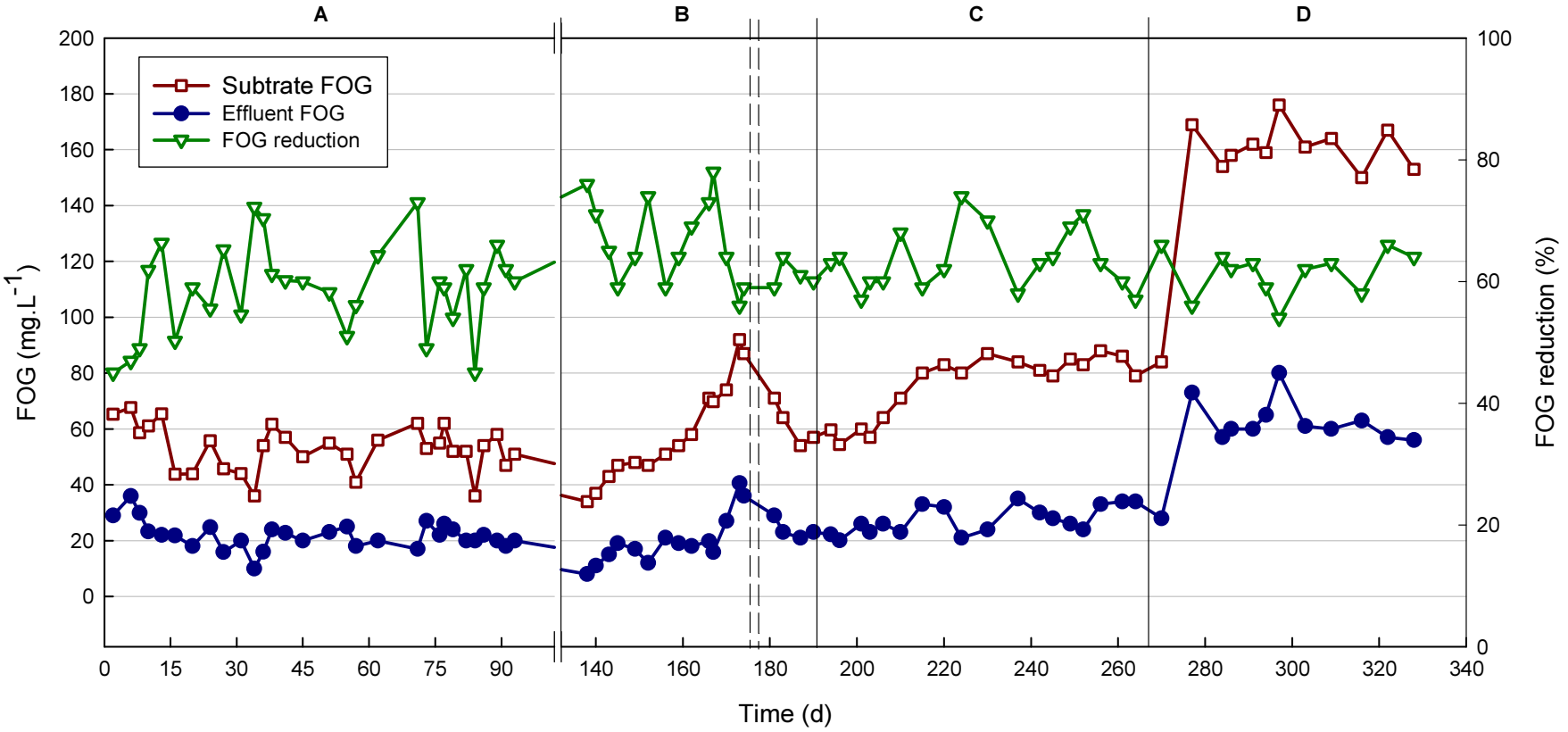


**Figure 4.4** Substrate FOG and Effluent FOG in the UASB reactor treating a FOG-reduced GDWW during the trial.

of GDWW diluted with reactor effluent. The reactor effluent is a source of alkalinity and by diluting the GDWW with effluent aided the reactor in maintaining a sufficient alkalinity. Substrate COD was initially adjusted to 2 000 $mg.L^{-1}$ and progressively increased to 5 000 $mg.L^{-1}$ by day 51 (Fig. 4.2). The full-scale Wellington UASB reactor functions at a 4:18 (GDWW:effluent) ratio. If it is considered that raw GDWW has a COD ranging from 20 000 to 25 000 $mg.L^{-1}$ and the reactor's reduction efficiency is maintained at 90%, operating a lab scale UASB reactor at the same ratio would relate to a substrate COD of *ca.* 5 500 $mg.L^{-1}$. Therefore, by day 60 the substrate COD concentration was readjusted and maintained at *ca.* 5 500 $mg.L^{-1}$ until day 109. Initially with the onset of the trial the COD reduction decreased from 70% to 60% where it remained stable until day 15 with increasing substrate COD being applied. As the substrate COD exceeded a concentration of 4 000 $mg.L^{-1}$ to reach 5 000 $mg.L^{-1}$ (day 12 to day 50) an improved COD reduction is observed, increasing from 60 to 75%. Operating at a COD load of *ca.* 5 500 $mg.L^{-1}$ the COD reduction remained in the range of 80% until day 109, ranging from 75 to 85%. A high effluent pH was measured during the initial part of phase A and as a result the substrate pH was not adjusted during days 0 to 34, resulting in inconsistent effluent pH measured (Fig. 4.3). Decreasing effluent pH resulted in lowered substrate pH and it was decided to adjust the substrate pH to 7.50 from day 35, effluent pH remained above 7.40 without any major changes visible.

With substrate COD remaining in the region of 5 500 $mg.L^{-1}$ it was decided to adjust the substrate pH to similar conditions experienced in the full-scale reactor. Changing a reactor parameter too quickly or in combination with another parameter (sudden increase in substrate COD) might result in a lowered overall efficiency. From day 60 to 68 substrate pH was decreased from 7.50 to 7.00. Substrate pH at 7.00 was maintained until the end of phase A (day 109). The effluent pH remained stable, ranging from 7.20 to 7.50, until day 98 when an increase was measured.

Measured alkalinity was higher than the optimum range for operation UASB reactors (1 000 – 3 000 $mg.L^{-1}$) as recommended by Gerardi (2000), ranging from 3 125 to 4 250 $mg.L^{-1}$ (day 0 – 30). This high alkalinity level resulted in the high effluent pH measured initially, however, with an ever increasing COD load applied and substrate pH not adjusted the alkalinity levels and effluent pH began to decrease. When substrate pH was adjusted to 7.50 by day 35 alkalinity remained more stable, ranging from 2 500 to 2 875 $mg.L^{-1}$ (day 35 to 60). When the pH was decreased to 7.00 alkalinity levels began to show less

stability, ranging from 2 000 to 2 875 mg.L$^{-1}$ (day 60 – 109), though remaining in the optimum range.

High initial levels of VFA decreased from 1 225 mg.L$^{-1}$ (day 0) to 153 mg.L$^{-1}$ (day 40) when a sudden increase was measured from day 51. The VFA's are the most important intermediate during anaerobic digestion and the sudden increase experienced on day 55 may be as a result of a kinetic uncoupling between the acid producers (acidogens) and consumers (acetogens and methanogens) as suggested by different researchers (Gerardi, 2003; Pind *et al.*, 2003; Arbeli *et al.*, 2006). However, with the sudden increase in VFA all other parameters measured remained stable except for the COD reduction, decreasing from 80 to 70%. The effluent pH during this part of the phase remained stable due to ability of the high alkalinity to buffer the VFA build up. Gerardi (2003) suggested a VFA to alkalinity ratio of 0.1 – 0.2 for stable reactor conditions, whereas a ratio more than 0.5 are symptomatic of poor reactor conditions. For most of phase A the ratio varied from 0.17 to 0.25. The high system alkalinity buffered the increase in VFA levels and prevented any rapid change in pH.

It was expected that substrate FOG would increase with substrate COD. However, there is no observable correlation between the two parameters. In reality, a decrease in substrate FOG was measured. Substrate FOG decreased from an initial 65 (substrate COD *ca.* 2 500 mg.L$^{-1}$) to 51 mg.L$^{-1}$ (substrate COD *ca.* 5 500 mg.L$^{-1}$) (Fig. 4.4). Effluent FOG also decreased from an initial 30 (Effluent COD *ca.* 500 mg.L$^{-1}$) to 22 mg.L$^{-1}$ (effluent COD *ca.* 1 000 mg.L$^{-1}$). The FOG reduction efficiency showed no improvement in stability, ranging from 45 to 73% throughout phase A. The substrate consisting of effluent and FOG-reduced GDWW, both a source of FOG, would over time measure higher FOG concentration if the reactor's degradation ability did not improve. However, as can be seen a decrease in substrate FOG and effluent FOG was measured over time. Thus, the biomass had to acclimatise to the wastewater's characteristics. As an observable increase in FOG reduction efficiency was not observed, it can be assumed that the FOG degradation is a rate limiting step and although the biomass have developed a degree of resistance the overall degradation efficiency have not increased considerably.

Due to an unforeseeable problem with the GDWW supply the reactor was not fed from day 109 to day 130. During this starvation period it was speculated that the system would use any substrate, in this case FOG, encapsulating the biomass as an energy source. It was also

speculated that this induced starvation might result in a possible increase in FOG degrading organisms within the biomass.

*Phase B (day 131 – 192)*

The aim of phase B was to test whether the UASB reactor could efficiently operate at COD load of *ca.* 10 000 $mg.L^{-1}$ and pH of 6.50. The UASB reactor was restarted on day 131, after 22 days of inactivation, at a COD load *ca.* 4 000 $mg.L^{-1}$. The substrate COD was increased to 5 500 $mg.L^{-1}$ by day 160 (Fig. 4.2). During this time the COD reduction efficiency decreased from 90 to 68%. Substrate pH maintained at 7.50 from day 131 to 137, by day 138 the pH was decreased to 7.00 followed by another decrease to attain 6.50 by day 153 (Fig. 4.3). From day 161 the substrate COD was progressively increased and by day 174 a concentration of 8 000 $mg.L^{-1}$ was attained. During this time the effluent COD increased from *ca.* 1 800 to 4 590 $mg.L^{-1}$, with a paralleled decrease in COD reduction (diminishing from 70 to 43%).

From Fig. 4.3 it can be observed that the combined lowering of the pH to 6.50 (from day 153) and the increased substrate load (from day 160) resulted in the decrease of alkalinity. Alkalinity decreased from 3 200 (day 134) to 1 500 $mg.L^{-1}$ (day 174). The increased COD may have resulted in the inability of the biomass to effectively breakdown the components to $CH_4$ and $CO_2$, thus, resulting in VFA build up. The reactor's buffer capacity (alkalinity) was being exhausted resulting in acidification and subsequent pH fluctuations. The VFA levels increased from 475 $mg.L^{-1}$ (day 139) to 800 $mg.L^{-1}$ (day 155) (effluent pH and substrate COD at 7.11 and *ca.* 6 400 $mg.L^{-1}$, respectively) and further increased to 1 120 $mg.L^{-1}$ (day 174) (effluent pH and substrate COD at 6.05 and *ca.* 8 110 $mg.L^{-1}$, respectively).

Deteriorating reactor efficiency can also be seen in the FOG removal capacity of the UASB reactor. Substrate FOG increased from 37 to 58 $mg.L^{-1}$ with the restart of the reactor at day 131 to day 160 (Fig. 4). During this part of phase B the removal efficiency ranged from 16 to 20 $mg.L^{-1}$. From day 161 to day 175 the substrate FOG increased from 58 to 92 $mg.L^{-1}$, since initial reactor start up and this was the highest substrate FOG concentration recorded. Effluent FOG concentrations doubled from 20 (day 161) to 40 $mg.L^{-1}$ (day 176). It can be seen from Fig. 4.4 that although the FOG removal efficiency remained in the range of 50 % and above, the fluctuation was more frequent and intense than that of phase A. In order to avoid reactor failure it was decided to flush the reactor with N&P solution and to suspend

feeding to improve the state of the reactor. It can be assumed that the deteriorating reactor efficiency experienced by day 174 was as a result of the combined increased in substrate COD load and decreased substrate pH. No substrate was fed from day 175 to 178 in an attempt to prevent the reactor's efficiency from deteriorating even further.

By day 179 the substrate COD and pH had been adjusted to the same levels used at the start of phase B. Substrate COD and pH were adjusted to 4 500 $mg.L^{-1}$ and 7.50, respectively. By decreasing the COD load the biomass could effectively degrade any excess intermediates in and around the biomass. The COD reduction efficiency improved from a poor 43% (day 174) to 75% (day 189). Increasing the substrate pH from 6.50 to 7.50 also improved system stability. The higher pH favours the methanogens and acetogens, thus increasing the consumption rate of the intermediaries (VFA's). Effluent pH increased with the higher substrate pH, ranging from 7.50 to 7.80. The increased substrate pH and effluent pH was reflected with improved alkalinity and decreased VFA levels. Before the commencement of the recovery part of phase B (day 174) the alkalinity was measured at 1 500 $mg.L^{-1}$ and VFA at 1 120 $mg.L^{-1}$. These parameters improved with alkalinity increasing to 3 125 $mg.L^{-1}$ (day 187) and VFA decreasing to 900 $mg.L^{-1}$ (day 186). The FOG reduction efficiency, however, did not change after adjusting the substrate with reduction efficiency remaining in the region of 56 to 61% (day 174 to 187).

It can be concluded from phase B that changing two parameters of substrate feed, especially as complex as GDWW, can result in a possible reactor failure. Increased COD load added pressure on to the system to uphold the degradation pathways of GDWW. Build-up of intermediaries such as VFA's and LCFA added pressure to uphold reactor alkalinity and further inhibition of microorganisms. Lowering of substrate pH and decreased reactor alkalinity did little to prevent deteriorating efficiency from accelerating. As a consequence, the biomass was unable to uphold the changing operational conditions and resulted in decreased efficiency which might have led to reactor failure if the feed was not readjusted.

*Phase C (day 193 – day 270)*

As phase B did not successfully accomplished the objectives of achieving a loading rate of *ca.* 10 000 $mg.L^{-1}$ in combination with a lowered substrate pH it was decided to re-evaluate whether a loading rate of 10 000 $mg.L^{-1}$ can be maintained by the UASB reactor whilst

substrate pH was kept at 7.50. If successful the full scale reactor could be modified to treat higher strength wastewater successfully.

By the start of phase C (day 193) the substrate COD was *ca.* 5 500 $mg.L^{-1}$ (Fig. 4.2). The substrate COD was maintained at *ca.* 5 500 $mg.L^{-1}$ until day 215 as COD reduction remained stable, ranging from 77 to 85%. The increase in substrate COD commenced on day 216 and by day 232 substrate COD had increased to *ca.* 8 000 $mg.L^{-1}$. The COD reduction efficiency also improved during this part, ranging from 80 to 90%. From day 232 until day 270 the substrate COD was progressively increased to 10 000 $mg.L^{-1}$.

As substrate COD continued to increase to 10 000 $mg.L^{-1}$ the reduction efficiency decreased from 90 (day 231) to 70% (day 248). The decrease in COD reduction efficiency was mirrored by observable spikes in the effluent pH, as a result of lowered alkalinity and increased VFA levels (Fig. 4.3). Effluent pH increased to 7.40 (day 233) before lowering to 7.00 (day 242) showing signs of instability in the reactor. During the same time there is a mirrored decrease in alkalinity as well as a rapid increase in VFA levels, as was experienced in phases A (day 45 to 60) and B (day 140 to 160). Alkalinity decreased from 3 000 to 2 450 $mg.L^{-1}$ whereas VFA increased from 410 to 630 $mg.L^{-1}$. These spikes cannot be attributed to substrate pH as this was kept constant for phase C at 7.50. During this time there was an increase in FOG reduction (Fig. 4). The FOG reduction improved from 55% (day 238) to 60% (day 242). It is possible that the increase in FOG degradation might have resulted in the spiking of VFA levels which led to pH fluctuations and decreased COD reduction. The addition of N&P solution improved alkalinity levels of the reactor and VFA levels decreased as a consequence. COD reduction started to improve from day 248, reaching an maximum removal efficiency of 94% by day 270. An increase in substrate FOG concentration was observed with the increasing COD load (from day 215) although the system was able to successfully degrade the FOG, however, reduction efficiency never exceeded 70% (Fig. 4.4).

Phase C proved successful as the substrate pH was kept constant whilst substrate COD was increased. The system did, however, struggle at times to maintain efficiency as COD increased resulting in lowered reduction efficiency and effluent pH fluctuations. However, sustaining the pH buffer proved pivotal for the successful attainment of *ca*. 10 000 $mg.L^{-1}$ COD. Build of intermediaries did occur but the system was able to buffer this effectively and degrade to $CH_4$ and $CO_2$.

*Phase D (day 271 – day 330)*

During phase D the reactor was continued to operate at a COD load of *ca*. 10 000 $mg.L^{-1}$ whilst the aim was to successfully decrease substrate pH to 6.50. After exceeding the desired substrate COD of 10 000 $mg.L^{-1}$ by day 270, the COD load was kept in the region of *ca*. 10 000 – 12 000 mg $COD.m^{-3}.d^{-1}$ until day 330. The substrate pH was gradually decreased from 7.50 to 6.50 from day 280 until day 295 (Fig. 4.3). During this adjustment all other parameters were closely monitored. Alkalinity levels during the pH decrease remained in the optimum range, ranging from 2 500 to 2 750 $mg.L^{-1}$ (Fig. 4.3). VFA levels did, however, visibly increase from 500 to 770 $mg.L^{-1}$ as substrate pH decreased (Fig. 4.3). A slight lowering of the effluent pH was also measured due to the ever changing alkalinity and VFA levels in the reactor, however, effluent remained above 7.0. Once the substrate pH had been lowered to 6.50, both the COD load and pH was kept constant until the end of the trial. Alkalinity and VFA levels became more stable. Alkalinity ranged between 2 000 and 2 500 $mg.L^{-1}$, whereas VFA stabilised in the range of 750 and 900 $mg.L^{-1}$. Effluent pH remained stable above 7.0 until the end of phase D.

The rapid increase in substrate FOG (Fig. 4) could be related to the increased COD load being applied as well as a decrease in the pre-treatment effectiveness. It was found that the untreated GDWW posed problems during pre-treatment. The thawing of GDWW, stored for more than three months, from -18ºC resulted in the formation of large aggregates which results in a less effective flocculation and subsequent FOG removal during pre-treatment. However, this problem will not occur if implemented in full-scale where GDWW is continuously produced, without the need of storage, and the pre-treatment system and subsequent UASB reactor will operate in conjunction with one another. The increase in substrate FOG resulted in higher effluent FOG measure, however, reduction efficiency remained in the region of 55% to 66% (Fig. 4.4). This reduction efficiency is comparable to the phase A to C (Fig. 4.4). Phase D proved successful as the COD load was kept constant whilst lowering substrate pH. Degradation pathways proceeded efficiently and there was no added pressure from an increase in substrate.

Various parameters were measured during the trial excluding COD, FOG, pH, VFA and alkalinity. These parameters included biogas production ($L.d^{-1}$), biogas composition (% $CH_4$) and total soluble solids (TSS). Data of the parameters were taken on different days during the trial and can be summarised in Table 4.4.

**Table 4.4** A summary of the UASB efficiency parameters monitored while treating GDWW. Data were taken at the end of each phase during the trial.

| | | Day 109 | Day 174 | Day 278 | Day 330 |
|---|---|---|---|---|---|
| **$pH_{substrate}$** | | 7.05 | 6.55 | 7.52 | 6.5 |
| **$pH_{effluent}$** | | 7.22 | 6.05 | 7.29 | 7.14 |
| **Alkalinity** | (mg $CaCO_3.L^{-1}$) | 2 750 | 1 500 | 2 600 | 1 935 |
| **VFA** | ($mg.L^{-1}$) | 463 | 1120 | 502 | 754 |
| **% $CH_4$** | | 67 | 51 | 61 | 64 |
| **Biogas** | ($L.d^{-1}$) | 6.0 | 4.6 | 8.9 | 18.0 |
| **OLR** | ($kgCOD.m^{-3}.d^{-1}$) | 5.5 | 8.1 | 10.0 | 10.0 |
| **$COD_{substrate}$** | ($mg.L^{-1}$) | 5 500 | 8 110 | 10 250 | 10 060 |
| **$COD_{effluent}$** | ($mg.L^{-1}$) | 1 120 | 4 590 | 615 | 1 006 |
| **% COD reduction** | | 80 | 44 | 94 | 90 |
| **$TSS_{substrate}$** | ($g.L^{-1}$) | 0.32 | 0.8 | 0.57 | 0.72 |
| **$TSS_{effluent}$** | ($g.L^{-1}$) | 0.15 | 0.48 | 0.24 | 0.3 |
| **$FOG_{substrate}$** | ($mg.L^{-1}$) | 51 | 92 | 169 | 153 |
| **$FOG_{effluent}$** | ($mg.L^{-1}$) | 20 | 40 | 73 | 56 |
| **% FOG reduction** | | 61 | 57 | 57 | 65 |

### Granule activity test

It was initially proposed that the long term exposure of the UASB reactor to GDWW would lead to the acclimatisation of the UASB granules to the wastewater as well as resulting in a possible microbial population shift. This acclimatisation and population shift would result in the biomass degrading GDWW more effectively compared to that of unacclimatised biomass. This increase could be determined by measuring the granule activity in the form of cumulative methane production (Fig. 4.5) and methane production rate (Fig. 4.6).

The improved reactor performance (as COD reduction) and biogas production together with an increase in activity on day 331 over day 0 could be an indication of a possible acclimatisation or the possibility of a microbial population shift happening.

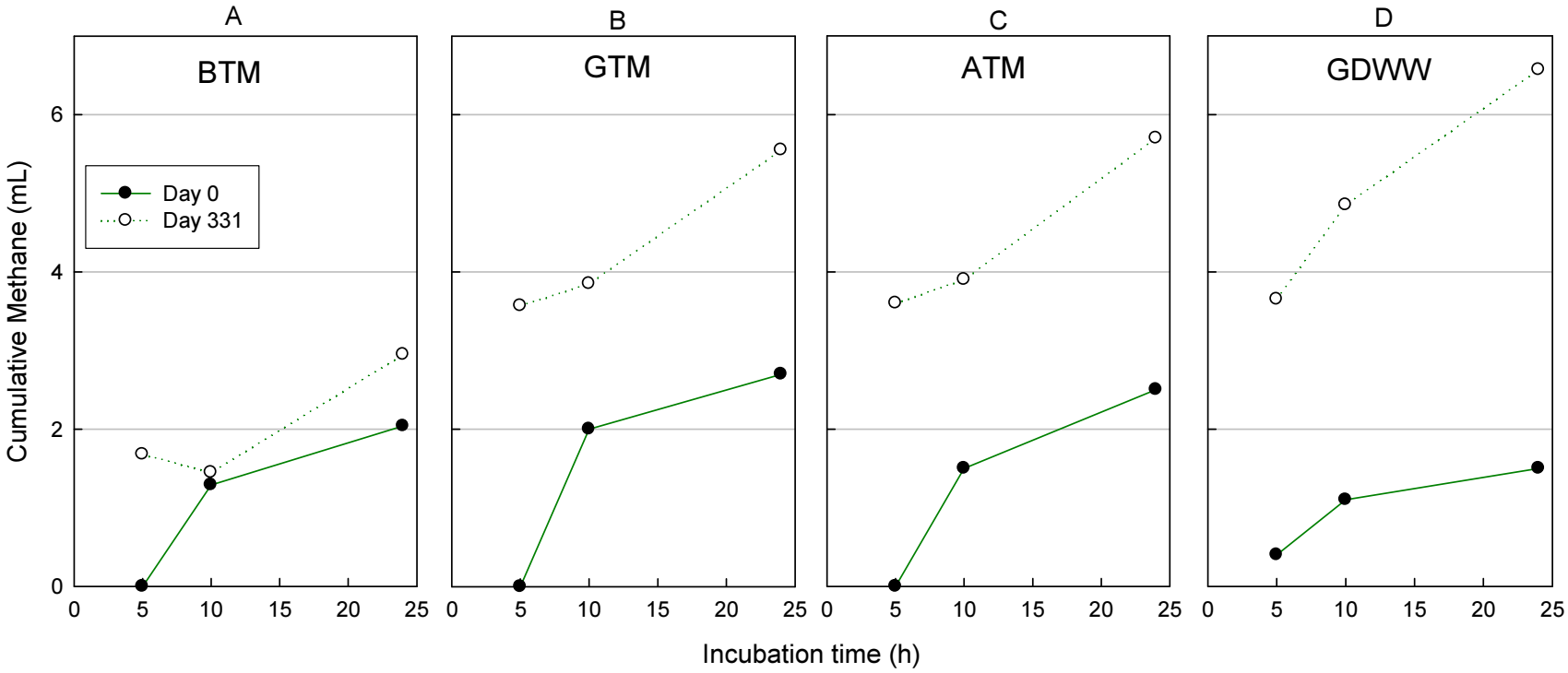


**Figure 4.5** Cumulative methane production of the UASB granules used to seed the reactor (day 0) and of UASB granules at the end of the trial (day 331), after incubation in BTM, GTM, ATM and GDWW.

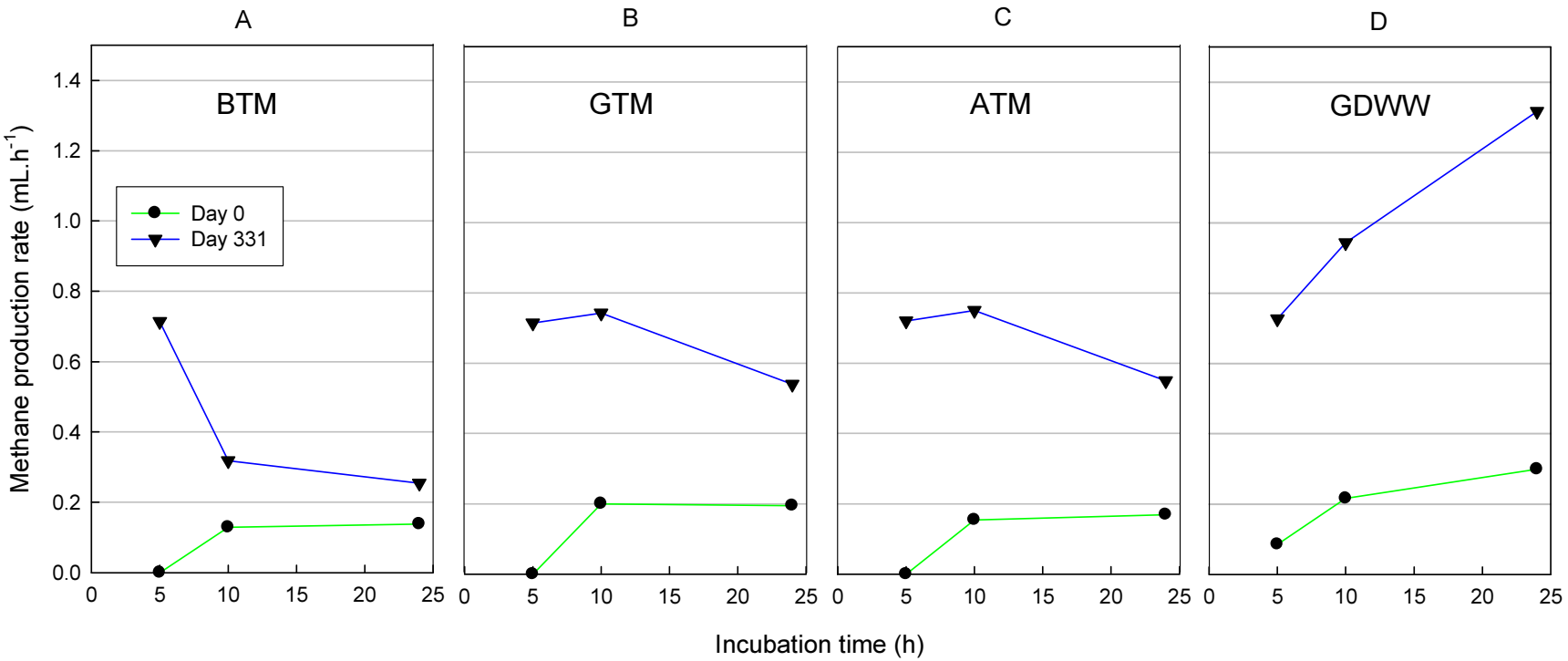


**Figure 4.6** Methane production rate of the UASB granules on day 0 and day 331 after 24 h incubation in BTM, GTM, ATM and GDWW.

*BTM*

The BTM is the control media, no specific group of microorganisms are given an advantage in the biomass, thus it is a measurement of the overall granule activity. It can be seen from Fig. 4.5A that the UASB granules, after exposure to GDWW for 331 days, produced higher methane volumes compared to the initial (day 0) granules. Cumulative methane production, for the initial granules, increased from 0 to 2 mL over the 24h activity test (Fig. 4.5A). The cumulative methane production for the 331 day old granules, however increased from 1.7 to 2.95 mL over the 24h test. It can also be seen (Fig. 4.6A) that the 331 day old granules had a much higher methane production rate at the start of the activity test (0.7 $mL.h^{-1}$), which decreased over the 24h test period, probably due to substrate depletion. The rate for the initial granules was 0 $mL.h^{-1}$ after 5h and only increased to 0.16 $mL.h^{-1}$.

*GTM*

The addition of glucose to the GTM favours the conditions of the acidogens, the largest trophic group in the UASB granules (Gerardi, 2003). It can be seen from Fig. 4.5B that UASB granules, after exposure to GDWW for 331 days, continued to show higher methane volumes compared to that of initial (day 0) granules. Cumulative methane production, for the initial granules, increased from 0 to 2.7 mL over the 24h activity test (Fig. 4.5B). The cumulative methane production for the 331 day old granules, however increased from 3.6 to 5.6 mL over the 24h test. It can also be seen (Fig. 4.6B) that the 331 day old granules had a much higher methane production rate at the start of the activity test (0.7 $mL.h^{-1}$), which decreased over the 24h test period, probably due to substrate depletion. The rate for the initial granules was 0 $mL.h^{-1}$ after 5h and only increased to 0.2 $mL.h^{-1}$.

*ATM*

Adding acetic acid to the ATM favours the conditions for acetoclastic methanogens, the group are responsible for the conversion of acetic acid to methane (Gerardi, 2003). It can be seen from Fig. 4.5C that the UASB granules, after 331 days of activity, produced higher methane volumes compared to that of initial (day 0) granules. Cumulative methane production, for the initial granules, increased from 0 to 2.5 mL over the 24h activity test (Fig. 4.5C). The cumulative methane production for the 331 day old granules, however increased from 3.6 to 5.7 mL over the 24h test. It can also be seen (Fig. 4.6C) that the 331 day old granules had a much higher methane production rate at the start of the activity test

(0.7 $mL.h^{-1}$), which decreased over the 24h test period, probably due to substrate depletion. The rate for the initial granules was 0 $mL.h^{-1}$ after 5h and only increased to 0.18 $mL.h^{-1}$.

*FOG-reduced GDWW*

Whereas all other test media were made up with different minerals and compounds specifically to favour the conditions of certain microbial groups within the biomass GDWW is much more complex in composition, giving it a low biodegradability. It was decided to use FOG-reduced GDWW as a fourth test media to determine whether or not the biomass did acclimatise to this type of wastewater during the 331 days of exposure in the UASB reactor. Cumulative methane production, for the initial granules, increased from 0.5 to 1.4 mL over the 24h activity test (Fig. 4.5D). The cumulative methane production for the 331 day old granules, however increased from 1.5 to 6.6 mL over the 24h test. This is also the highest measured volume of all the test media, indicating biomass acclimatisation. It can also be seen (Fig. 4.6D) that the 331 day old granules had a much higher methane production rate at the start of the activity test (0.7 $mL.h^{-1}$), which increased over the 24h test period to 1.32 $mL.h^{-1}$. The long term exposure to FOG-reduced GDWW is indicative how the microbial consortium specialised to break down the complex wastewater. The production rate continued to increase as complex substrates were broken down resulting in an increased conversion rate to methane which was also the highest for all the test media. The rate for the initial granules was 0.09 $mL.h^{-1}$ after 5h and only increased to 0.3 $mL.h^{-1}$ after 24h.

## CONCLUSIONS

The aim of this study was to test the feasibility of UASB digestion of FOG-reduced GDWW. The first objective successfully attained reactor start-up and maintained an organic loading rate similar to that of a full-scale UASB reactor from a local distillery. The second objective investigated the ability of the UASB reactor's efficiency in terms of COD and FOG removal whilst OLR was increased and a lowered substrate pH. After an initial deterioration of reactor efficiency (after OLR was increased in combination with lowered substrate pH) the UASB reactor was able to successfully treat FOG-reduced GDWW at an OLR of *ca.* 10 $kgCOD.m^{-3}.d^{-1}$ and substrate pH at 6.50. Lastly, a granule activity test was performed and granules from UASB reactor treating FOG-reduced GDWW were compared to initial seed granules. These granules showed increased activity over seed granules in

terms of methane production rate and cumulative methane production. The FOG-reduced GDWW test media was indicative to what degree the microbial consortia from the granules have specialised to breakdown complex wastewater, such as GDWW. However, FOG removal efficiency never improved to such an extent as expected during the UASB reactor feeding suggesting that the complexity of breaking down GDWW have rate limiting steps. Further investigation is required to improve efficiency.

It was essential to remove sufficient amounts of FOG and TSS from the GDWW during pre-treatment before a subsequent UASB treatment. Implementation of a coagulation/flocculation-centrifugation treatment proved to be sufficient in order to reduce the FOG content of GDWW before the onset of UASB treatment. This method of pre-treatment will, however, have to be investigated in-depth to ascertain whether improved FOG reduction can be reached as well as the impact thereof in a full-scale operation. Table 4.5 provides a proposed summary of the UASB reactor parameters required to efficiently treat FOG-reduced GDWW as per the results obtained from this investigation.

**Table 4.5** Proposed optimum operational conditions for a UASB reactor treating FOG-reduced GDWW.

| Parameter | | |
|---|---|---|
| **$COD_{substrate}$** | ($mg.L^{-1}$) | 5 500 |
| **HRT** | (hr) | 24 |
| **OLR** | ($kgCOD.m^{-3}.d^{-1}$) | 5.50 |
| **$pH_{substrate}$** | | 7.00 |
| **$FOG_{substrate}$** | ($mg.L^{-1}$) | < 55 |
| **$Alkalinity_{substrate}$** | (mg $CaCO_3.L^{-1}$) | 2 000 – 2 500 |
| **$TSS_{substrate}$** | ($g.L^{-1}$) | < 0.40 |

## REFERENCES

Akunna, J.C. & Clark, M. (2000). Performance of a granular-bed anaerobic baffled reactor (GRABBR) treating whisky distillery wastewater. *Bioresource Technology*, **74**, 257-261.

Alves, M., Vieira, J., Pereira, R., Pereira, M. & Mota, M. (2001). Effects of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors. Part II: Oleic acid toxicity and biodegradability. *Water Research*, **35**, 264 - 270.

APHA (1998). *Standard methods for the examination of water and wastewater*, 20th ed. Pp. 2:26-28, 22:54-58, 24:86-87, 24:149-150, 145:113-117, 145:134-139. Washington D. C.

Arbeli, Z., Brenner, A. & Abeliovich, A. (2006). Treatment of high-strength dairy wastewater in an anaerobic deep reservoir: analysis of the methanogenic fermentation pathway and the rate-limiting step. *Water Research*, **40**, 3653-3659.

Cammarota, M.C. & Freire, D.M.G. (2006). A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresource Technology*, **97**, 2195-2210.

Cavaleiro, A.J., Alves, M.M. & Mota, M. (2001). Microbial and operational response of an anaerobic fixed bed digester to oleic acid overloads. *Process Biochemistry*, **37**, 387-394.

Chernicharo, C.d.L. (2007). *Anaerobic Reactors,* Pp. 1 - 184. IWA Publishing.

Deepak, L.J. (1998). High rate anaerobic treatment of industrial wastewater in tropics. *International Journal of Science and Technology*, **3**, 1 - 7.

Forday, W. & Greenfield, P.F. (1983). Anaerobic digestion. *Effluent and Water Treatment Journal*, **23**, 405 - 409.

Gao, M., She, Z. & Jin, C. (2007). Performance evaluation of a mesophilic (37 °C) upflow anaerobic sludge blanket reactor in treating distiller's grains wastewater. *Journal of Hazardous Materials*, **141**, 808-813.

Gerardi, M.H. (2003). *The Microbiology of Anaerobic Digesters,* Pp. 1 - 192. Wiley.

Gie, L. (2007). Effect of pre-treated distillery wastewater on upflow anaerobic sludge blanket granules and reactor efficiency. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Goodwin, J.S. & Stuart, J.B. (1994). Anaerobic digestion of malt whisky distillery pot ale using upflow anaerobic sludge blanket reactors. *Bioresource Technology*, **49**, 75-81.

Goodwin, J.S., Wase, D.J. & Forster, C.F. (1990). Effects of nutrient limitation on the anaerobic upflow sludge blanket reactor. *Enzyme and Microbial Technology*, **12**, 877-884.

Guida, M., Mattei, M., Della Rocca, C., Melluso, G. & Meriç, S. (2007). Optimization of alum-coagulation/flocculation for COD and TSS removal from five municipal wastewater. *Desalination*, **211**, 113-127.

Hogg, R. (2000). Flocculation and dewatering. *International Journal of Mineral Processing*, **58**, 223-236.

Koster, I. & Cramer, A. (1987). Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids. *Applied and environmental microbiology*, **53**, 403-409.

Lettinga, G., van Velsen, A.F.M., Hobma, S.W., de Zeeuw, W. & Klapwijk, A. (1980). Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnology and Bioengineering*, **22**, 699-734.

Lu, C., Yeh, A.C. & Lin, M.-R. (1995). Treatment of high-strength organic wastewaters using an anaerobic rotating biological contactor. *Environment International*, **21**, 313-323.

McLachlan, T. (2004). Integration of a combined UASB-ozonation treatment system for cellular effluent degradation. MSc in Food Science Thesis, University of Stellenbosch, South Africa.

Mendes, A. & Castro, H. (2005). Effect on the enzymatic hydrolysis of lipids from dairy wastewater by replacing Gum Arabic emulsifier for sodium chloride. *Brazilian Archives of Biology and Technology*, **48**, 135-142.

Meyer, L., Hugo, A., Britz, T., De Witt, B. & Lategan, P. (1983). Temperature control for laboratory scale anaerobic digesters. *Water SA*, **9**, 79-80.

O' Kennedy, O.D. (2000). Application of biogranules in the anaerobic treatment of distillery effluents. In: MSc in Food Science Thesis, University of Stellenbosch.

Pereira, M., Pires, O., Mota, M. & Alves, M. (2002). Anaerobic degradation of oleic acid by suspended and granular sludge: identification of palmitic acid as a key intermediate. *Water Science & Technology*, **45**, 139 - 144.

Pind, P., Angelidaki, I. & Ahring, B. (2003). Dynamics of the anaerobic process: effects of volatile fatty acids. *Biotechnology and Bioengineering*, **82**, 791-801.

Sarkar, B., Chakrabarti, P., Vijaykumar, A. & Kale, V. (2006). Wastewater treatment in dairy industries—possibility of reuse. *Desalination*, **195**, 141-152.

Sigge, G. & Britz, T. (2007). UASB treatment of a highly alkaline fruit-cannery lye-peeling wastewater. *Water S. A.*, **33**, 275-278.

Tiwari, M., Guha, S., Harendranath, C. & Tripathi, S. (2006). Influence of extrinsic factors on granulation in UASB reactor. *Applied Microbiology and Biotechnology*, **71**, 145-154.

Walsdorff, A., Van Kraayenburg, M. & Barnardt, C. (2005). A multi-site approach towards integrating environmental management in the wine production industry. *Sustainable Viticulture and Winery Wastes Management*, **51**, 61-69.

Zhou, Y., Liang, Z. & Wang, Y. (2008). Decolorization and COD removal of secondary yeast wastewater effluents by coagulation using aluminum sulfate. *Desalination*, **225**, 301-311.

# Chapter 5

## ACCLIMATISATION OF UASB GRANULES TO FOG-REDUCED GRAIN DISTILLERY WASTEWATER FOLLOWING A STRATEGIC FEEDING APPROACH


### SUMMARY

The efficiency and acclimatisation of a UASB reactor fed with FOG-reduced grain distillery wastewater (GDWW) was investigated. FOG-reduced GDWW was fed to a laboratory scale UASB reactor (2.3 L) using a strategic dosing approach. The dosing approach consisted of several feeding and starvation cycles. Improved average biogas production was observed during the feeding (0.26 to 11.3 $L.d^{-1}$) and starvation (1.8 to 4.2 $L.d^{-1}$) cycles as higher loading rates were obtained. After the completion of the strategic feeding the UASB reactor was continuously fed at an organic loading rate of *ca.* 5 $kgCOD.m^{-3}.d^{-1}$. The chemical oxygen demand (COD) reduction efficiency improved from 70 to 80% but the fats, oils and grease (FOG) reduction remained in the region of 60%. Granule activity tests done on days 0, 215 and 279 showed improved UASB granule activity to GDWW with operation time in terms of methane production rate and cumulative methane production.


### INTRODUCTION

Distilleries worldwide are producing large volumes of wastewater (15 L of wastewater produced for each litre of ethanol), with severe environmental pollution/implications if not treated (Satyawali & Balakrishnan, 2007; Mohana *et al.*, 2009). Grain distillery wastewater (GDWW) is a waste product produced during grain whisky distillation and is considered a high strength wastewater due to its unique characteristics. The GDWW is rich in nutrients, proteins and fats, oils and grease (FOG). The high protein content and total soluble solids (TSS), low pH and high temperature give GDWW a low biodegradability, which if not treated correctly, may have detrimental effects on the environment (Mendes & Castro, 2005; Cammarota & Freire, 2006; Chipasa & Mdrzycka, 2008).

The upflow anaerobic sludge blanket (UASB) reactor operates as a suspended growth system (without the use of any packing material) with the active biomass being held in suspension by hydraulic design (Deepak, 1998; Tiwari *et al.*, 2006). The anaerobic nature of the system results in the biological conversion of the waste into biogas, consisting of methane ($CH_4$), carbon dioxide ($CO_2$) and a small amount of biomass (Gavrilescu, 2002; Gerardi, 2003). The main groups of microorganisms responsible for anaerobic digestion are the acidogens, acetogens and methanogens (Forday &

Greenfield, 1983; Gavrilescu, 2002; Chernicharo, 2007). It is important that the process of anaerobic digestion must be as efficient as possible to avoid any accumulation of intermediates, which may disturb the system's performance (Gavrilescu, 2002). The use of the UASB reactor to treat distillery wastewater has been well documented by different researchers. Gao *et al.* (2007) successfully treated GDWW achieving up to 97.3% COD reduction at an OLR between 5 and 48 $kgCOD.m^{-3}.d^{-1}$ and with a hydraulic retention time (HRT) ranging between 11 and 82 h. In contrast, Goodwin (1994) was able to successfully treat GDWW at a loading rate of 15 $kgCOD.m^{-3}.d^{-1}$. Uzal *et al.* (2003) used a two-stage UASB system to reduce up to 93% COD from distillery wastewater and further increased the COD reduction up to 99% during a subsequent aerobic treatment step.

The high lipid content of GDWW is, however, often associated with problems during biological treatment, especially anaerobic treatment (Cavaleiro *et al.*, 2007). These operational problems are a result of the accumulation of lipids onto the microbial aggregates by adsorption, precipitation and entrapment (Cavaleiro *et al.*, 2007). The adsorption of lipids onto the biomass can alter the sludge's ability to settle and can lead to sludge bed washout. Accumulation can also create a physical barrier that hinders the transfer of substrates and products (Cavaleiro *et al.*, 2001; Cavaleiro *et al.*, 2007; Chipasa & Mdrzycka, 2008). Long chain fatty acids (LCFA), intermediates of lipid metabolism, have been reported to have inhibitory effects on acetoclastic methanogens and acetogens although the mechanism of action is not completely understood (Koster & Cramer, 1987; Rinzema *et al.*, 1994; Mendes & Castro, 2005; Miranda *et al.*, 2005). Yet the anaerobic digestion of LCFA is possible if the reactor is continuously fed, well mixed and sudden overloading is avoided (Rinzema *et al.*, 1994). Hwu *et al.* (1998) proposed a bi-absorption model of LCFA degradation. The LCFA's are adsorbed from the aqueous phase onto the solid phase during which no degradation occurs. The LCFA's are desorbed leading to an increase in the LCFA concentration in the aqueous phase and after a period the LCFA's are re-absorbed and degraded. The concentration of the LCFA will determine the lag phase involved before complete degradation occurs (Hwu *et al.*, 1998). The LCFA are degraded via the β-oxidation pathway to acetic acid by the acetogens (Cavaleiro *et al.*, 2007). Cavaleiro *et al.* (2007) also studied the accumulation and biodegradation of LCFA during UASB treatment of oleate rich wastewater and found that during cycles of feeding the system was able to develop a specialised microbial consortium able to effectively treat this type of wastewater. The development of the specialised consortium made the system more resistant to toxicity from LCFA compared to unacclimatised biomass (Alves *et al.*, 2001).

It is important to develop a specialised consortuim of microorganisms capable of efficiently degrading a certain type of wastewater. The improved degradation ability would avoid kinetic uncoupling between the different microbial groups responsible for digestion. In Chapter 4 it was shown that a UASB reactor could successfully treat FOG-reduced GDWW during a long-term operation. In Chapter 4, the UASB reactor was fed over a period of 331 days and several operational parameters were attained and evaluated. By the end of the trial the UASB reactor was able to successfully treat a COD and FOG load of *ca.* 10 000 and 160 $mg.L^{-1}$, respectfully. However, it was found that the UASB reactor was sensitive to COD load increases, resulting in poor system stability on several occasions until stability was re-established. To enhance the overall system stability of the UASB reactor treating GDWW would require a more controlled start-up that could possibly result in an enhanced GDWW degradation with a specialised UASB biomass.

The aim of this investigation is to step-wise increase the COD and FOG degradation capabilities of a lab-scale UASB reactor treating FOG-reduced GDWW. Firstly, reactor start-up with the FOG-reduced GDWW will be evaluated by following a strategic feeding approach, including cycles of feeding and subsequent cycles of starvation. Each of the feeding cycles will be aimed at attaining a higher COD load until the desired COD load is attained. In a second phase, the UASB reactor will be fed continuously at the maximum attained COD to evaluate the operational stability of the UASB under these conditions. Finally, a granule activity test was performed on seed granules and compared to the activity of UASB granules used in this trial.

## MATERIALS AND METHODS

### Strategic feeding

This trial was divided into two phases. Phase A involved feeding the laboratory-scale UASB reactor with FOG-reduced GDWW. The strategic feeding approach of Phase A involved feeding the UASB reactor in 7 cycles, as summarised in Table 5.1. The UASB reactor used for this study (reactor 2) was operated in an identical manner to the reactor used in Chapter 4 of this thesis (reactor 1) in terms of feeding intervals, re-circulation speed and temperature. This was done so as to allow a performance comparison between an “acclimatised” and a non-acclimatised reactor. During each cycle a period of 20 d was used for feeding and 10 d for starvation (except for the 2$^{nd}$ feeding and starvation, in which a 30 d feeding and 15 d starvation was used, due to unforeseeable problems with the GDWW supply). All feeding cycles were started at an initial GDWW COD strength of *ca.*

500 mg.L$^{-1}$. Each feeding cycle, however, attained a higher substrate COD by the end of that particular feeding cycle. During each feeding cycle the substrate COD was increased on a daily basis until the attainment of the required COD. The feeding cycles continued until a FOG-reduced GDWW COD of *ca.* 5 000 mg.L$^{-1}$ was attained at the end of the 7$^{th}$ cycle. Figure 5.1 shows a representation of the cycles followed during Phase A.

Following the completion of each feeding cycle the UASB reactor was flushed with an N & P solution (500 mg.L$^{-1}$ urea ($(NH_2)_2CO$) and 500 mg.L$^{-1}$ di-potassium hydrogen orthophosphate ($K_2HPO_4$)) before the period of starvation. During this starvation no substrate was fed as it was postulated that any degradation activity would be as a result of the biomass utilising substances accumulated on the biomass as a source of energy. These substances would potentially include FOG and LCFA that had encapsulated the granules.

After completing Phase A the reactor was continuously fed with a substrate consisting of FOG-reduced GDWW, which was diluted with reactor effluent to the required COD of *ca*. 5 000 mg.L$^{-1}$. Dilution with the reactor effluent was done to retain the alkalinity formed during digestion to buffer reactor pH and prevent any rapid changes from occurring.

**Table 5.1** A summary of the strategic feeding strategy followed during Phase A. By the end of each cycle a higher substrate COD was attained resulting in an increased daily substrate COD being applied.

| Cycle | Time (d) | Attained COD (mg.L$^{-1}$) | Average daily COD increase (mg.L$^{-1}$.d$^{-1}$) |
|---|---|---|---|
| **1** | 0 – 30 | 1 500 | 50 |
| **2** | 31 – 75 | 2 500 | 67 |
| **3** | 76 – 105 | 3 000 | 125 |
| **4** | 106 – 135 | 3 500 | 150 |
| **5** | 136 – 165 | 4 000 | 175 |
| **6** | 166 – 195 | 4 500 | 200 |
| **7** | 196 – 215 | 5 000 | 225 |

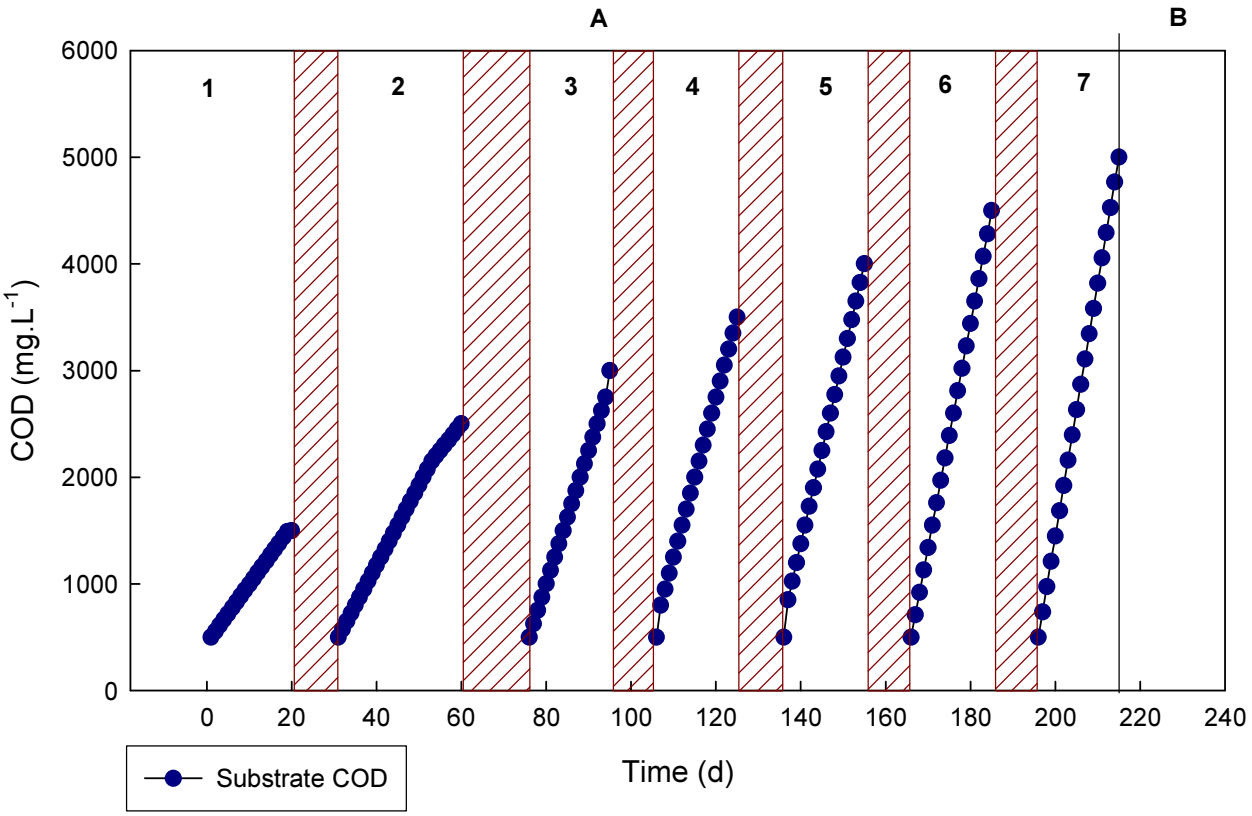


**Figure 5.1** Increased COD loading during the feeding/starvation cycles of the UASB reactor treating FOG-reduced GDWW. The shaded areas represent the periods of starvation.

*Reactor start-up*

Granular sludge was obtained from a full-scale UASB reactor treating winery distillery wastewater (WDWW) in Wellington (South Africa) and was used to seed the lab-scale UASB reactor. Start-up was initiated by flushing the UASB reactor with water containing 500 $mg.L^{-1}$ urea ($(NH_2)_2CO$) and 500 $mg.L^{-1}$ di-potassium hydrogen orthophosphate ($K_2HPO_4$) for a period of 24 h. Once the flushing had been completed the strategic feeding at 500 $mg.L^{-1}$ COD commenced.

**GDWW and Pre-treatment**

The GDWW used for this trial was received from a distillery in Wellington, South Africa, from September 2008 until June 2009. The GDWW was stored in 25 L drums at –18ºC until required. Once required, a 25 L drum was allowed to thaw and was stored at 4ºC while in use. The GDWW underwent the coagulation/flocculation-centrifugation pre-treatment technique developed and reported in Chapters 3 and 4 of this thesis. The pre-

treatment included a flocculation step with 250 mg.L$^{-1}$ $FeCl_3$ (Chlorchem) followed by a centrifugation step to separate the heavy (solids) and the liquid fractions.

The coagulation/flocculation was achieved by means of adding 250 mg.L$^{-1}$ $FeCl_3$ to the GDWW followed by mixing at 130 rpm for 2 min on a shaker (Labcon). After coagulation/flocculation, 200 g of the GDWW was weighed in 250 mL centrifuge bottles and placed in the centrifuge (Beckman Coulter TJ-25). The GDWW was centrifuged for 10 min at 10 000 rpm (15 ºC). The solids (FOG and TSS rich) fraction was discarded. The liquid (FOG reduced GDWW) fraction after centrifugation was stored and served as the basis for the reactor substrate. FOG-reduced GDWW used during the strategic feeding cycles was stored at 4ºC and was diluted to the required COD before feeding the reactor.

The FOG-reduced GDWW was diluted with tap water to a calculated COD during the strategic feeding (Phase A). The pH was adjusted with 2 M potassium hydroxide (KOH) during the trial. During Phase $A_1$ the pH was adjusted to 7.50 and by the beginning of Phase $A_2$ it was adjusted to 8.00. The addition of 1 mL trace element solution (TES) to the reactor was done on a weekly basis to ensure nutritional stability of the microorganisms. Addition of 500 mg.L$^{-1}$ urea ($(NH_2)_2CO$) and 500 mg.L$^{-1}$ di-potassium hydrogen orthophosphate ($K_2HPO_4$) on a daily basis ensured an optimum C:N:P ratio within the UASB reactor during the strategic feeding cycles. After the completion of Phase A the reactor was continuously fed at a COD *ca.* 5 000 mg.L$^{-1}$ (Phase B).

**Analytical methods**

The analytical parameters that were measured in the substrate and reactor effluent included: pH, alkalinity (as [$CaCO_3$]), COD and FOG (APHA, 1998). Determination of FOG was modified from the APHA (1998) method as follows: wastewater samples (100 g) were acidified to pH 2 with hydrochloric acid (HCl) (2 M), weighed (50 g) and transferred to a separator funnel. Absolute Ethanol (96%) (100 mL), 40 mL n-hexane and diethyl ether (1:1) were added to the separator funnel and the sample was shaken vigorously and left to settle into layers. The bottom layer was drained and the top layer (FOG concentrate) was collected. The drained layer was extracted a further three more times with n-hexane : diethyl ether (1:1) to extract more oils from the sample. The cumulative solvent sample was distilled in a rotavap (Büchi Rotavapor R-114) at 60°C and the distilled sample was measured gravimetrically and quantified to mg.L$^{-1}$.

Biogas composition measurements were taken routinely from the UASB reactor during the trial. Composition was determined by injecting a 0.2 mL biogas sample into a gas chromatograph (GC) (Varian 3300) (Sigge & Britz, 2007). The GC was equipped with

a thermal conductivity detector, 2.0 m x 3.0 mm I.D. column filled with a hayesep Q (Supelco, Bellefonte, PA) 80/100 mesh. Helium was the carrier gas at a determined flow rate of 30 $mL.min^{-1}$ and the oven temperature was set at 55°C.

**Granule activity test**

It was initially proposed that a controlled feeding approach would induce a faster acclimatisation of the biomass to the GDWW without any associated operational problems occurring. A granule activity test was performed on day 0 (seed sludge), day 215 (completion of Phase A) and on day 279 (end of Phase B). An increase in activity of the granules on day 215 and 279 could be an indication of possible of acclimatisation of the biomass to GDWW. Cumulative biogas production and methane production rate were used as indicators of increased granule activity.

Activity tests were done as described by O'Kennedy (2000) and were used to portray the overall microbial activity of the granules and certain microbial groups within the granules. The test media used for each activity test included a basic test media (BTM), glucose test media (BTM), acetic test media (ATM) and FOG-reduced GDWW (Table 5.2). Overall granule activity was determined by BTM where no specific group of microorganisms are given an advantage. Acidogenic and methanogenic activity was determined by GTM whereas the activity of the acetoclastic methanogens was determined by ATM. The FOG-reduced GDWW test media was added to the activity test to determine the increase in activity when comparing the seed sludge to sludge with extensive exposure to GDWW. An increase in activity in GDWW could also be seen as an indication of acclimatisation.

During the initial activity test (day 0), 50 g of the sampled granules were incubated in a 250 mL Schott bottle at 35°C over 48 h in 150 mL activation media (Table 5.3). The activation media was decanted after 24 h and replaced with fresh activation media. After 48h of incubation was completed, duplicate granule samples (3 g) were placed in 20 mL glass vials for each specific test media. Each of the glass vials received 13 mL of the specific test media leaving a 6 mL headspace. The vials were sealed with a butyl septa and enclosed with a aluminium cap and incubated at 35°C for 24 h. After 5, 10 and 24 h incubation the biogas volume was recorded by using a free moving 10 mL syringe with a 12 gauge needle. Once the septa seal was punctured by the syringe, the biogas volume was measured after the piston had stopped moving. The syringe was removed from the vial and inserted into the GC where the biogas composition was determined. On day 215

and 279 the same activity test was performed on granules from the UASB reactor. In this instance no pre-activation of the UASB granules was carried out.

**Table 5.2** Composition of basic test media (BTM) (O' Kennedy, 2000).

| Compound | Concentration ($g.L^{-1}$) |
|---|---|
| Glucose | 2.0 |
| Di- potassium hydrogen orthophosphate ($K_2HPO_4$) | 1.0 |
| Potassium di-hydrogen orthophosphate ($KH_2PO_4$) | 2.6 |
| Urea | 1.1 |
| Ammonium chloride ($NH_4Cl$) | 1.0 |
| Sodium sulphide ($Na_2S \cdot 9H_2O$) | 0.1 |
| Magnesium Chloride ($MgCl_2 \cdot 6H_2O$) | 0.1 |
| Yeast extract | 0.2 |
| pH | 7.1 |

**Table 5.3** Composition of the different test media used to determine the activity of certain microbial groups (O' Kennedy, 2000).

| Test media | Microbial group |
|---|---|
| Basic test media (BTM) | Control |
| Glucose test media (BTM + 2.0 $g.L^{-1}$ glucose) (GTM) | Acidogens |
| Acetic test media (BTM + 1.0 $g.L^{-1}$ acetic acid) (ATM) | Acetoclastic methanogens |
| FOG-reduced GDWW (diluted to *ca.* 5 000 $mg.L^{-1}$ COD) | Control |

**Table 5.4** Composition of the activation media used during the activity tests (O' Kennedy, 2000).

| Compound | Concentration ($g.L^{-1}$) |
|---|---|
| Glucose | 1.0 |
| Di- potassium hydrogen orthophosphate ($K_2HPO_4$) | 0.5 |
| Urea | 0.5 |

## RESULTS AND DISCUSSION

### Operational efficiency of the UASB reactor treating FOG-reduced GDWW

*COD reduction, pH and Alkalinity*

During the feeding cycles, the COD concentration was regularly measured to verify whether the calculated substrate COD was correct. Cycle 1 showed an overall substrate COD increase from 500 to 1 500 mg.L$^{-1}$ (Fig. 5.2). During this cycle the COD reduction decreased from an initial 92 to 84% as the substrate COD was gradually increased. During this cycle the alkalinity remained relatively constant between 900 and 975 mg.L$^{-1}$ (Fig. 5.3). The fact that the substrate was prepared by diluting the FOG-reduced GDWW with water rather than reactor effluent (as is often done in practice) meant that the alkalinity within the reactor could not be effectively maintained. However, using water during dilution enabled better control of the gradual increase in substrate COD and FOG. Alkalinity levels remained in the range of 1 000 mg.L$^{-1}$ which is generally considered considered the minimum for stable reactor efficiency (Gerardi, 2003). Effluent pH decreased from 7.70 to 6.40 during cycle 1, possibly as a result of certain groups of microorganisms not being able to degrade certain intermediates, such as organic acids, rapidly enough. Thus, the build-up of these intermediates as well as a low reactor alkalinity resulted in a decrease in the buffer capacity, leading to a decrease in effluent pH.

Feeding Cycle 2 was given a 30 day feed and 15 day starvation sequence instead of the 20 and 10 day cycle, respectively. This was due to GDWW availability issues experienced by the supplier. During this cycle a higher substrate COD of *ca.* 2 500 mg.L$^{-1}$ was attained (Fig. 5.2). The effluent pH did not show any stability throughout the entire cycle, ranging between 6.20 and 7.20, which resulted in low alkalinity (in the region of 1 000 mg.L$^{-1}$) (Fig. 5.3). The COD reduction, however, remained at an efficiency of *ca.* 80% during this cycle (Fig. 5.2).

During Cycles 3 and 4 it can be seen that the pH decreased rapidly after feeding commenced (Fig. 5.3). Accumulation of organic acids and other intermediates within the system during the feeding cycle, as a result of the increased COD load being applied, and low alkalinity could have resulted in the sudden decrease of the system pH. Initial COD reduction remained above 80% by the start of Cycles 3 and 4, however, with ongoing feeding the efficiency dipped below 75% (Fig. 5.2). With the ever decreasing system pH it was decided to increase substrate pH to 8.00 from day 119 onwards (Cycle 4, Phase $A_2$ start) (Fig. 5.2 and 5.3) in an attempt to counter the impact of the low effluent pH.

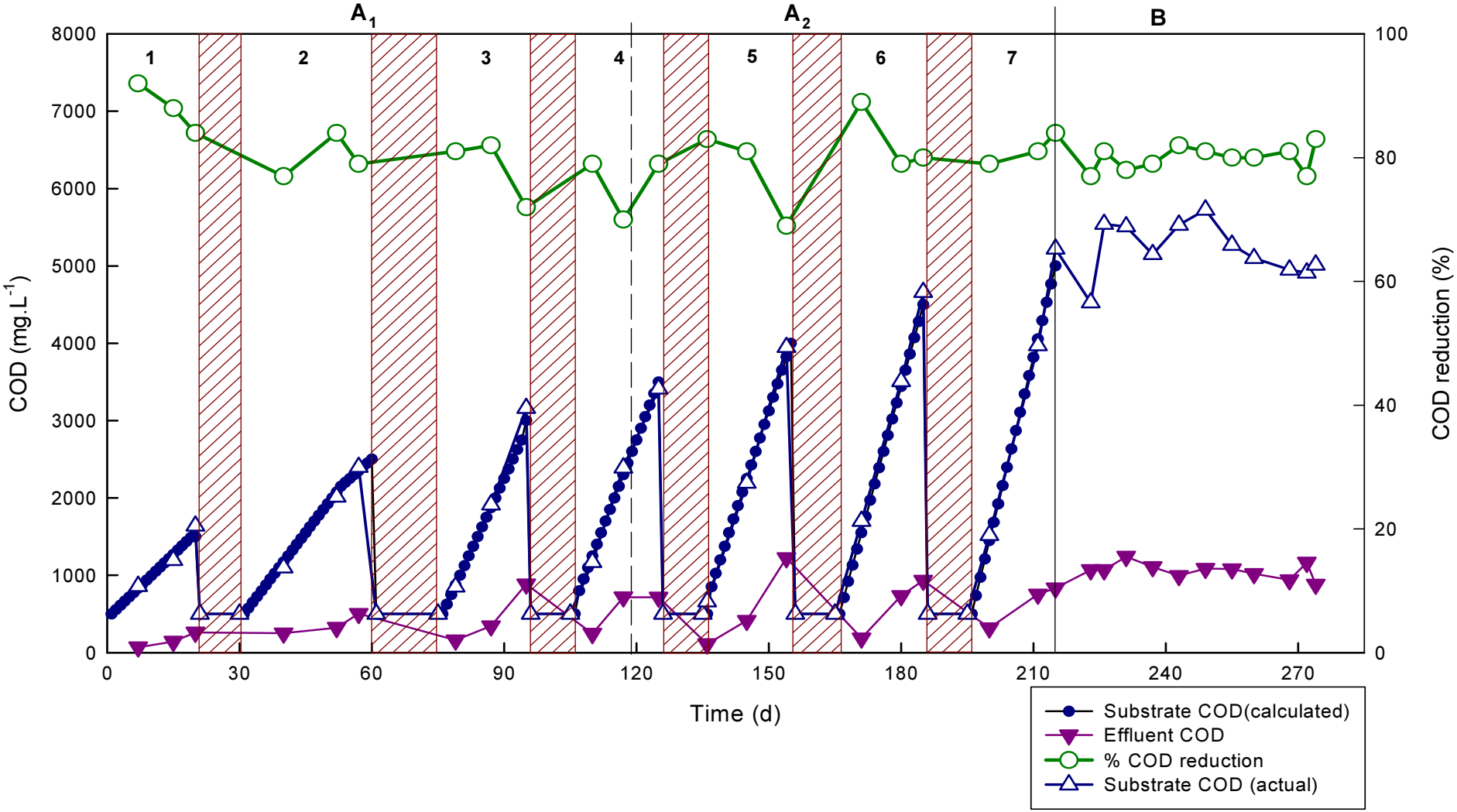


**Figure 5.2** Substrate COD (calculated), actual substrate COD, effluent COD and % COD reduction in the UASB reactor following a strategic dosing approach (Phase A) and a continuous substrate feed (Phase B). Substrate pH was at 7.50 and 8.00 for Phases $A_1$ and $A_2$, respectively.

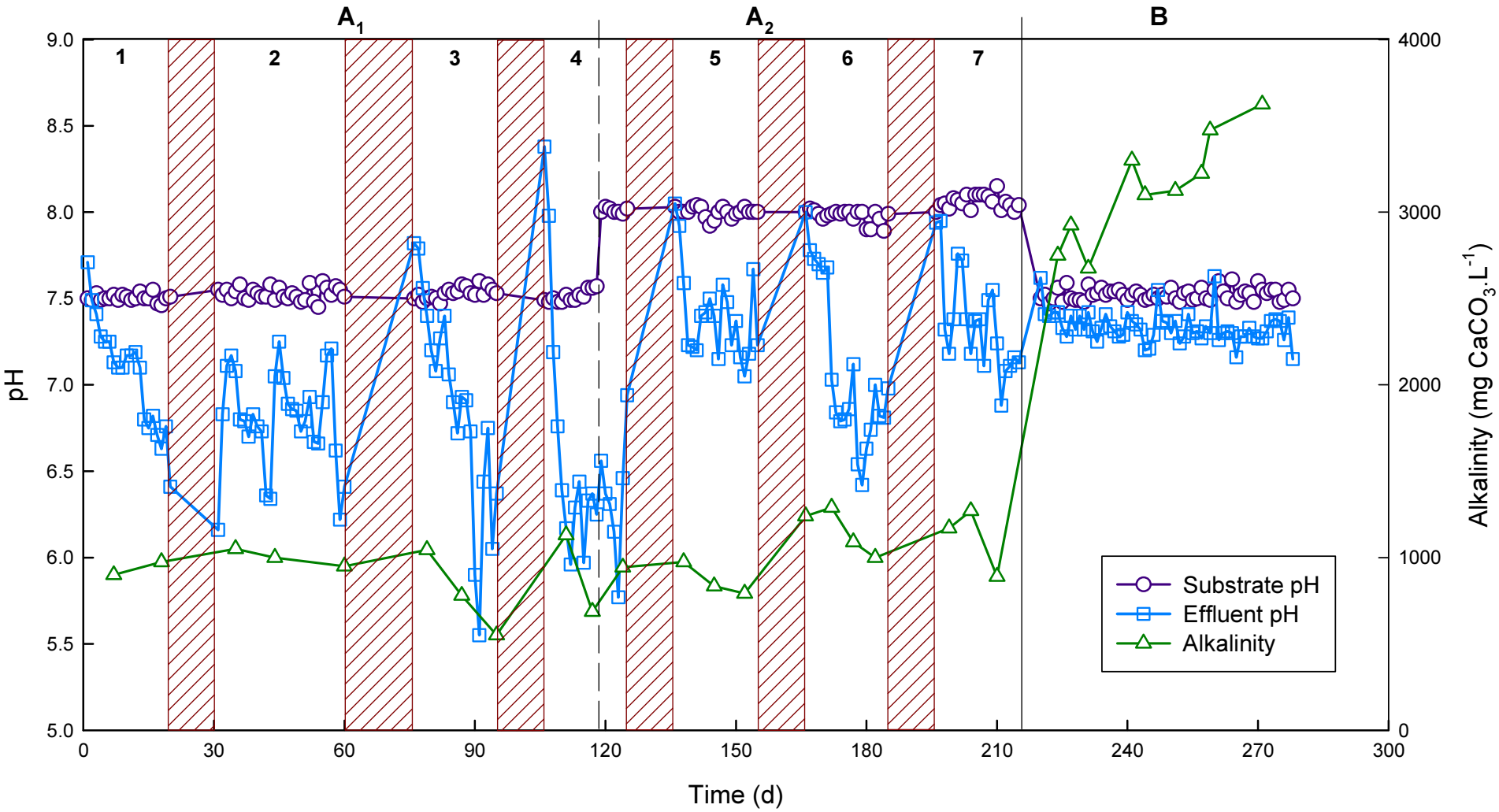


**Figure 5.3** Substrate pH, effluent pH and alkalinity experienced in the UASB reactor during the strategic feeding phase (Phase A) and continuous feeding treating FOG-reduced GDWW (Phase B).

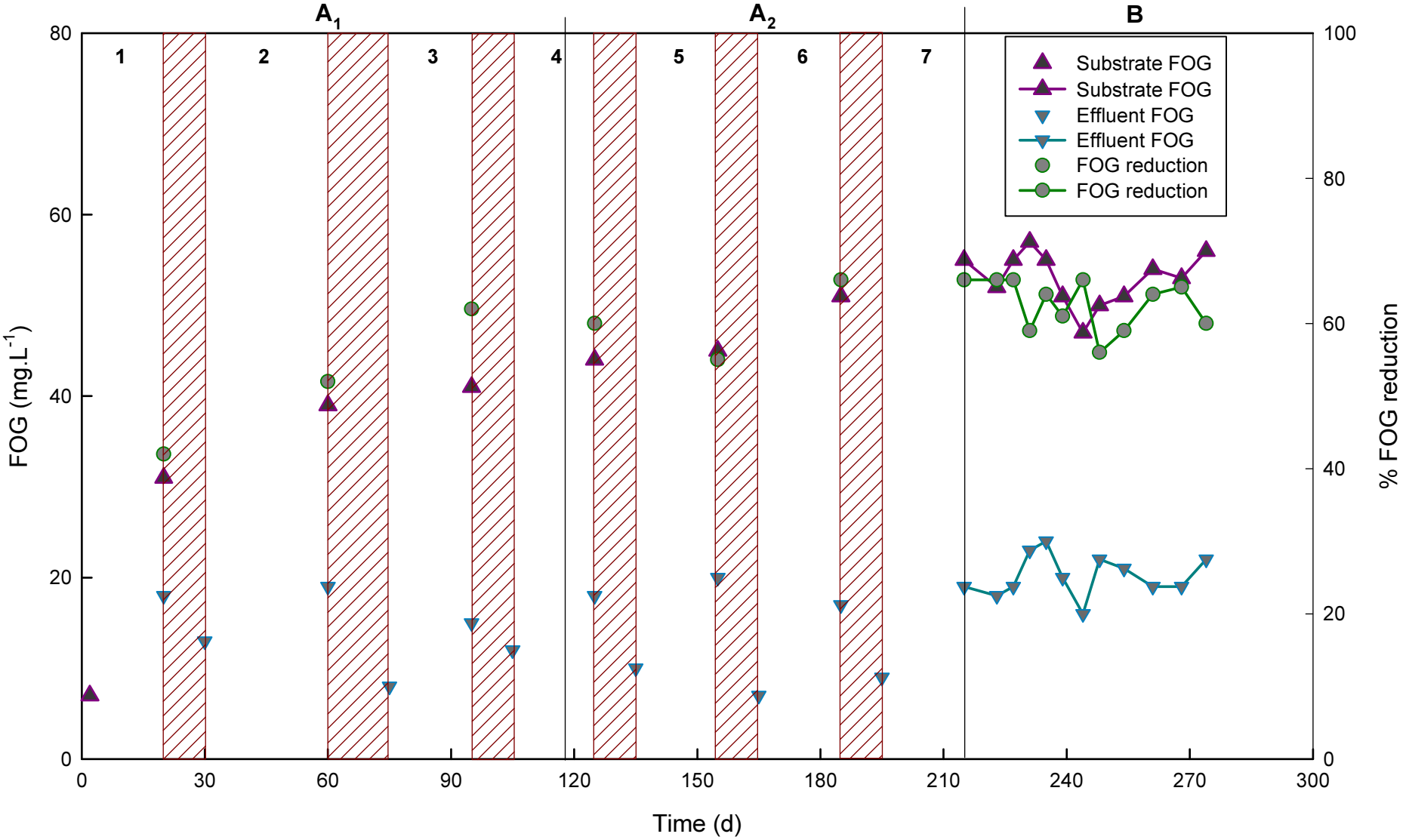


**Figure 5.4** Substrate FOG, effluent FOG and % FOG reduction in UASB reactor following the strategic feeding approach (Phase A) and continuous feed (Phase B).

After the increase in substrate pH there was an increase noted in alkalinity, with levels increasing from 687 (day 117) to 943 mg.L$^{-1}$ (day 125). However, effluent pH continued to decrease before increasing by the end of Cycle 4. The adjustment of the substrate pH did improve the COD reduction efficiency from 70 at day 117 to 79% by day 125. The increased substrate pH was maintained during cycle 5 and resulted in a higher and more stable effluent pH (Fig. 5.3). The higher substrate pH was kept until the end of Phase $A_2$ and the effluent pH remained between 7.0 and 7.5. Alkalinity levels decreased slightly from an initial 975 mg.L$^{-1}$ (cycle 5) to 800 mg.L$^{-1}$, but the COD reduction decreased from 83 to 69% during this cycle (Fig. 5.2).The lowered COD reduction efficiency could be ascribed to increasing substrate FOG concentration as a result of the higher substrate COD reached for every cycle. It is possible that the biomass had not yet fully acclimatised to the wastewater characteristics, resulting in inhibition and poorer reduction efficiency. During Cycle 6 a lower system pH was observed compared to Cycle 5, although it remained between 6.50 and 7.0 (Fig. 5.3). Alkalinity levels decreased slightly from an initial 1 050 to 950 mg.L$^{-1}$ during Cycle 6.

Although the initial COD reduction of 89% decreased slightly with the higher COD loading applied, reduction remained above 80% by the end of Cycle 6. This was an improvement in efficiency compared with the previous cycles. This improvement is most likely due to the higher alkalinity when compared to that of Cycles 3 and 4, however, effluent pH remained variable. By the start of Cycle 7, the reactor parameters started to show more stability. Effluent pH remained above 7.0 for most of Cycle 7 whereas the alkalinity remained in the region of 1 000 mg.L$^{-1}$. The COD reduction continued to show stability as efficiency remained in the region of 80%.

At the end of the 7$^{th}$ feeding Cycle the COD load reached was *ca.* 5 000 mg.L$^{-1}$ and instead of a follow-up starvation period, the reactor was continuously fed at this load from day 216 until the end of the trial (day 279) (Fig. 5.2). During this Phase the substrate consisted of FOG-reduced GDWW diluted with reactor effluent rather than water (to provide alkalinity) and the pH set at 7.50. This was maintained for the rest of the trial. It can be seen that the substrate COD ranged between 4 500 to 5 750 mg.L$^{-1}$ (Fig. 5.2). The reactor effluent used to dilute the GDWW also contributed to the total COD making control of the final substrate COD more difficult and resulting in slight variations of substrate COD (Fig. 5.2). The COD reduction efficiency ranged from 75 to 85% as effluent COD varied from 900 to 1 200 mg.L$^{-1}$. The COD reduction efficiency remained stable until the end of Phase B. Alkalinity continued to increase to above 3 000 mg.L$^{-1}$ by the end of the trial, while effluent pH ranged from 7.3 to 7.5 (Fig. 5.3). Adequate alkalinity is very important to

avoid any sudden pH changes from occurring within the UASB reactor. A stable reactor requires alkalinity levels from 1 000 to 2 000 mg.$L^{-1}$ (Gerardi, 2003). It could be seen that when alkalinity levels were low in the reactor, poor efficiency with regards to COD reduction and effluent pH were recorded (Cycles 3 and 4). If alkalinity levels were kept in the optimum range during the strategic feeding Phase it could have resulted in even greater efficiency as well as a possible shortening time required to fully acclimatise the biomass to FOG-reduced GDWW.

*FOG reduction*

Reactor FOG levels were measured at the end of each feeding and starvation cycle during the strategic feeding phase (Phase $A_1$ and $A_2$) and regularly during the continuous feeding phase (Phase B). As a higher substrate COD was readied during each feed cycle a higher substrate FOG was obtained as well. Substrate FOG levels increased from an initial 7 (day 1) to 31 (end of Cycle 1) to 55 mg.$L^{-1}$ (end of Cycle 7) (Fig. 5.4). Effluent FOG, measured at the end of each feeding cycle, remained relatively stable throughout (between 17 and 19 mg.$L^{-1}$) whereas measurements taken at the end of each starvation cycle showed a decrease during Phase A. From this it was assumed that the reactor's FOG degrading ability did increase. The FOG reduction increased from an initial 42 (Cycle 1) to 66% by the end of Cycle 7. During Cycles 4 and 5 there is a slight decrease in the % FOG reduction. Lower pH and alkalinity measured during these cycles could have resulted in the build-up of FOG and subsequent intermediates. This resulted in the poor COD and FOG reduction efficiency. However, increasing the substrate pH resulted in increased reduction efficiency in the subsequent cycles. By the end of Cycles 6 and 7 it can be seen that % FOG reduction improved to 66% as the reactor's effluent pH increased, COD reduction remained stable and alkalinity levels increased.

During starvation cycles it can be assumed that the biomass utilised the FOG as a carbon source, once the reactor was flushed with an N & P solution after each feeding cycle to remove excess COD. As FOG reduction increased throughout the feeding part of each cycle it can be seen that FOG levels were reduced during each starvation. The reduction efficiency varied for each starvation and there is no clear indication of improved reduction by the system as it varied between 20 and 65% (Fig. 5.4). The variation in starvation reduction could be related to the reactor conditions (pH, alkalinity and VFA levels) by the end of the feeding cycle.

During Phase B substrate FOG levels remained in the region of 55 mg.$L^{-1}$ and never exceeded 60 mg.$L^{-1}$. The FOG reduction efficiency remained in the region of 60 to

65% until the end of Phase B. Substrate consisting of FOG-reduced GDWW and effluent (source of alkalinity) and a stable system pH resulted in improved reactor conditions and subsequent stable FOG reduction efficiency.

*Biogas production*

During Phase A, the increasing COD load applied during each cycle led to more substrate being degraded which in turn resulted in the increase in biogas production. The average biogas production increased from an initial 0.26 $L.d^{-1}$ (cycle 1) to 11.3 $L.d^{-1}$ by cycle 7 (Table 5.5). There is also a slight increase in the average methane ($CH_4$) composition in the biogas with ongoing exposure time. Cycle 6 showed the highest percentage of methane composition (93%) during biogas production, however, cycle 7 had the highest methane yield with regards to average biogas production. The high methane yield might suggest that possible acclimatisation of the biomass resulting in increased methane production from the FOG, however, a high pH can also lead to a higher methane percentage in the biogas measurements (Gerardi, 2003).

The average biogas content was measured during starvation periods to give an indication of any further activity. Due to the fact that the reactor had been flushed with a N & P solution any activity would be either as a result of digestion of intermediates formed or the use of the accumulated FOG surrounding the granule. The FOG may also be responsible for the entrapment of any other substances surrounding the granules. The average biogas production during the starvation cycles increased from 0.4 $L.d^{-1}$ to 4.2 $L.d^{-1}$ (cycle 6). The increase in biogas production during starvation cycles provides a possible indication of degradation of any substances like FOG, which have accumulated within the system. In Fig. 5.4 it can be seen that there is a decrease in system FOG at the end of each starvation cycle and this together with the increase in biogas production during the starvation cycles may be indicative of increased activity of FOG degrading microorganisms.

*Continuous feeding versus a strategic feeding approach*

To determine whether the strategic feeding approach increased the overall performance of this reactor it was compared to the efficiency of the reactor used in Chapter 4. The reactor in Chapter 4 was fed continuously over a period over 331 days. Two periods of operation of the reactor in Chapter 4 were used for comparison. Firstly, days 45 to 90 were compared, as the COD load during this time was similar to that applied during the strategic feeding approach.

**Table 5.5** Average biogas production during the strategic feeding approach and continuous feeding of the UASB reactor treating FOG-reduced GDWW.

| Cycle | Substrate COD attained ($mg.L^{-1}$) | Average biogas production ($L.d^{-1}$) during feeding | Average % $CH_4$ in biogas | Average $CH_4$ yield ($L\ CH_4.d^{-1}$) | Average biogas production ($L.d^{-1}$) during starvation |
|---|---|---|---|---|---|
| **1** | 1 500 | 0.26 | 69% | 0.18 | 0.4 |
| **2** | 2 500 | 0.45 | 70% | 0.32 | 0.7 |
| **3** | 3 000 | 1.16 | 72% | 0.83 | 1.8 |
| **4** | 3 500 | 4.0 | 77% | 3.08 | 1.3 |
| **5** | 4 000 | 4.4 | 77% | 3.38 | 4.7 |
| **6** | 4 500 | 8.0 | 93% | 7.44 | 4.2 |
| **7** | 5 000 | 11.3 | 81% | 9.2 | -* |
| **Continuous feed** | *ca.* 5 000 | 10.9 | 73% | 7.96 | -* |

* No starvation cycle

**Table 5.6** Comparison of UASB reactor parameters measured during this investigation (Reactor 2) and the UASB reactor investigation from Chapter 4 (Reactor 1).

| | Chapter 4 (Reactor 1) | Chapter 4 (Reactor 1) | Strategic feeding approach (Reactor 2) |
|---|---|---|---|
| **Operation period (d)** | 45 - 90 | **216 - 279** | **216 - 279** |
| **COD load ($mg.L^{-1}$)** | **5 500** | 5 000 – 10 000 | **5 000** |
| **% COD reduction** | 60 – 85 | 73 – 92 | 75 – 85 |
| **% FOG reduction** | 50 – 60 | 55 – 75 | 60 – 65 |
| **Substrate pH** | 7.0 | 7.50 | 7.50 |
| **Effluent pH** | 7.5 – 7.80 | 7.0 – 7.50 | 7.30 – 7.50 |
| **Alkalinity ($mg.L^{-1}$)** | 1 500 – 2 700 | 2 400 – 3 500 | 2 700 – 3 700 |
| **Average % $CH_4$** | 67% | 56% | 73% |
| **Average Biogas production ($L.d^{-1}$)** | 6 | 7 | 7.9 |

Secondly, the period of day 216 – 279 of both reactors was compared. The reactor in Chapter 4 was treating COD loads of 5 000 – 10 000 $mg.L^{-1}$ while the strategic feeding approach reactor had reached, and was maintained at *ca.* 5 000 $mg.L^{-1}$. The comparisons are summarised in Table 6.

In Fig. 5.5 both reactors are compared to one another in reduction efficiency, alkalinity and effluent pH. COD reduction efficiency remained relatively in the same range for both reactors at the different times and loading rates. It can be seen from Fig. 5.5 that Reactor 2, following the strategic feeding approach, showed more stable COD and FOG reduction range compared to that of Reactor 1 (Chapter 4). There is a minor increase noted in FOG reduction efficiency from Reactor 1 to Reactor 2. As was the case with COD reduction efficiency did Reactor 2 show improved stability over Reactor 1. The effluent pH measured for Reactor 2 was lower than that of Reactor 1 in both cases, however, it was still within the optimum ranged for a UASB reactor (between 6.50 and 7.50). The measured effluent pH was in the same region for Reactor 1 (7.30 to 7.70) and Reactor 2 (7.20 – 7.55) when substrate COD was in the same region, *ca.* 5 000 (Reactor 2) and 5 500 $mg.L^{-1}$ (Reactor 1), respectively. Effluent pH was more stable for Reactor 2 compared to Reactor 1 (7.05 to 8.00) in the same period (day 216 to 279). Alkalinity improved from Reactor 2 over Reactor 1 and is well within the optimum range (1 000 to 3 000 $mg.L^{-1}$) (Fig. 5.5). It can be assumed that the microbial consortia of both reactors did in fact acclimatise to the FOG-reduced GDWW's characteristics. However, following the strategic feeding approach there was improvement in overall stability of the reactor with regard to FOG and COD reduction, stable effluent pH, improved alkalinity and $CH_4$ production.

**Granule Activity Test**

The purpose of the activity test was to determine whether the UASB granules were able to successfully acclimatise to the FOG-reduced GDWW following the strategic feeding approach. An increased activity in the specific test media could be a confirmation of acclimatisation of the biomass. The activity was measured in the form of cumulative methane production (Fig. 5.6) and methane production rate (Fig. 5.7).

*BTM*

The basic test medium (BTM) determines the overall granule activity where no specific microbial group in the granule is favoured and this gives an indication of the overall activity

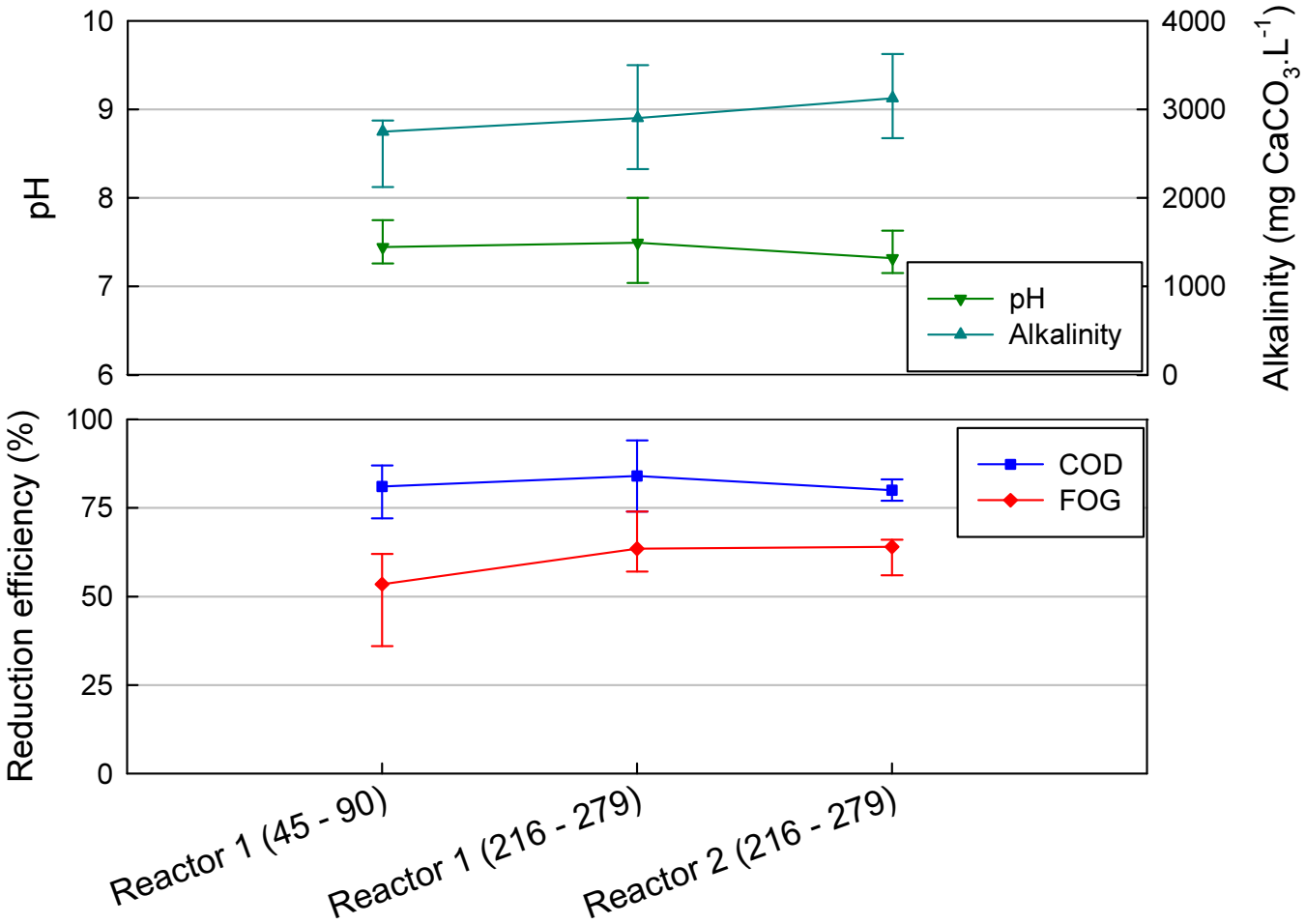


**Figure 5.5** COD reduction efficiency, FOG reduction efficiency, effluent pH and alkalinity – median comparisons between Reactor 1 (Chapter 4) and Reactor 2 (following a strategic feeding approach).

of the system. It can be seen from Fig. 6A that the UASB granules exposed to FOG-reduced GDWW during the strategic dosing phase and from the continuous feeding phase (day 215 and 279, respectively) produced higher methane volumes compared to the initial (day 0) granules. Cumulative biogas production after 24 h was initially 3.32 mL (day 0) (Fig. 5.6A). The cumulative methane production increased to 4.76 mL (day 215) and 5.80 mL (day 279), respectively, thus exhibiting an increased cumulative methane production by the acclimatised granules. The methane production rate (Sm) for the initial (day 0) granules only showed activity by 10 and 24 h, whereas granules from day 215 and 279 showed an initial production rate at 5 h and decreased afterwards (Fig. 5.7A). The methane production rate after 24h is also still higher for the acclimatised granules. High initial methane production (5 h) decreased by 10 and 24 h as the substrate was depleted by the acclimatised biomass.

*GTM*

The addition of glucose to the basic test medium (BTM), makes it a glucose-rich medium (GTM) favouring the conditions for the acidogens, the largest trophic group in the UASB granules. It can be seen from Fig. 5.6B that the UASB granules exposed to FOG-reduced GDWW during the strategic dosing phase and from the continuous feeding phase (day 215 and 279, respectively) produced higher methane volumes compared to the initial (day 0) granules. Initial (day 0) cumulative methane production was 4.68 mL (Fig. 5.6B). The cumulative biogas production increased to 9.21 mL (day 215) and 12.67 mL (day 279) respectively, thus exhibiting improved methane production by the acclimatised granules. Methane production rate (Fig. 5.7B) at 5 h shows more activity in granules acclimatised to FOG-reduced GDWW at day 215 and 279 compared to day 0. Methane production rate started to decrease after 10 h as substrate began to deplete.

*ATM*

The added acetic acid to ATM enhances the activity of the acetoclastic methanogens. This group are responsible for the conversion of acetic acid to methane. The UASB granules exposed to FOG-reduced GDWW following a strategic dosing phase and from continuous feeding phase (day 215 and 279, respectively) showed higher biogas production compared to initial (day 0) granules (Fig. 5.6C). Cumulative biogas production after 24h was initially 4.07 mL (day 0) (Fig. 5.6C).

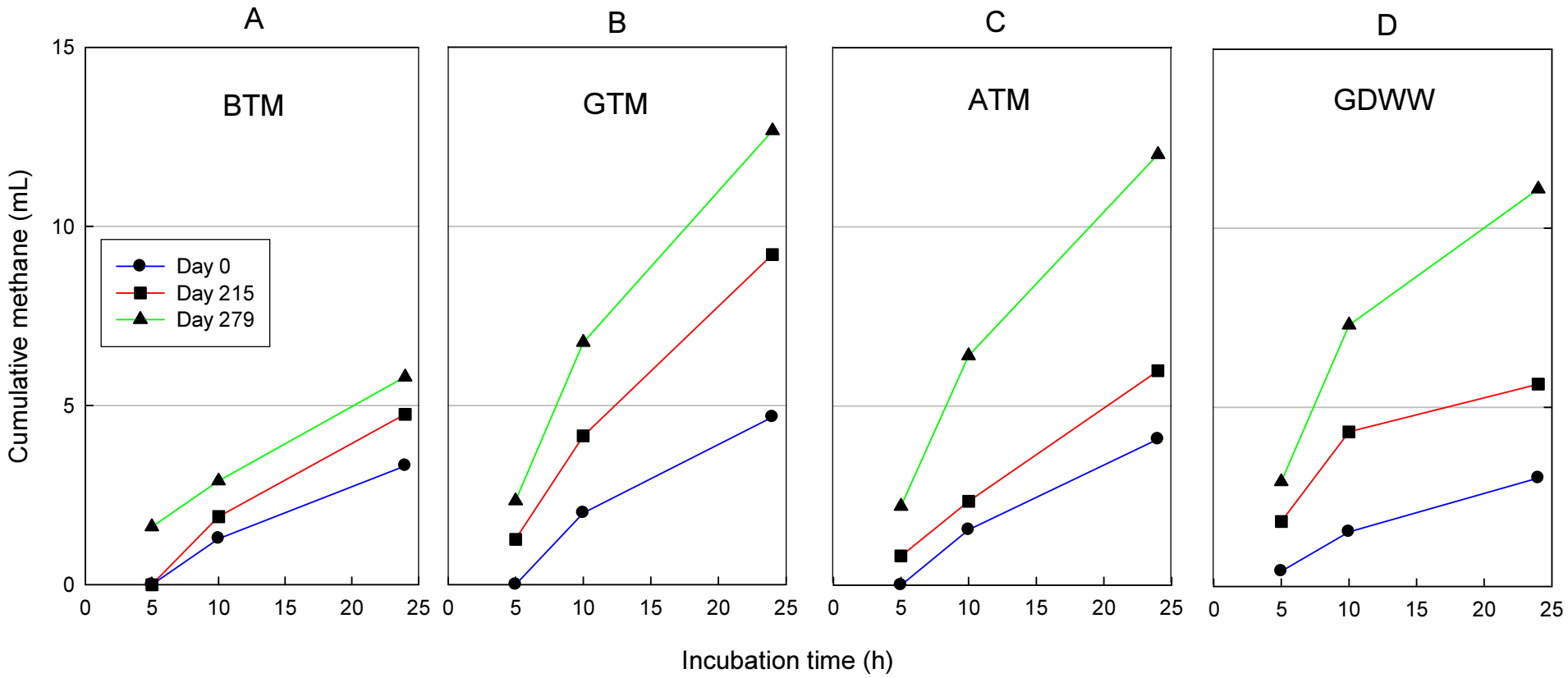


**Figure 5.6** Cumulative methane production of the UASB granules on day 0, day 215 and day 279 after incubation in BTM, GTM, ATM and GDWW.

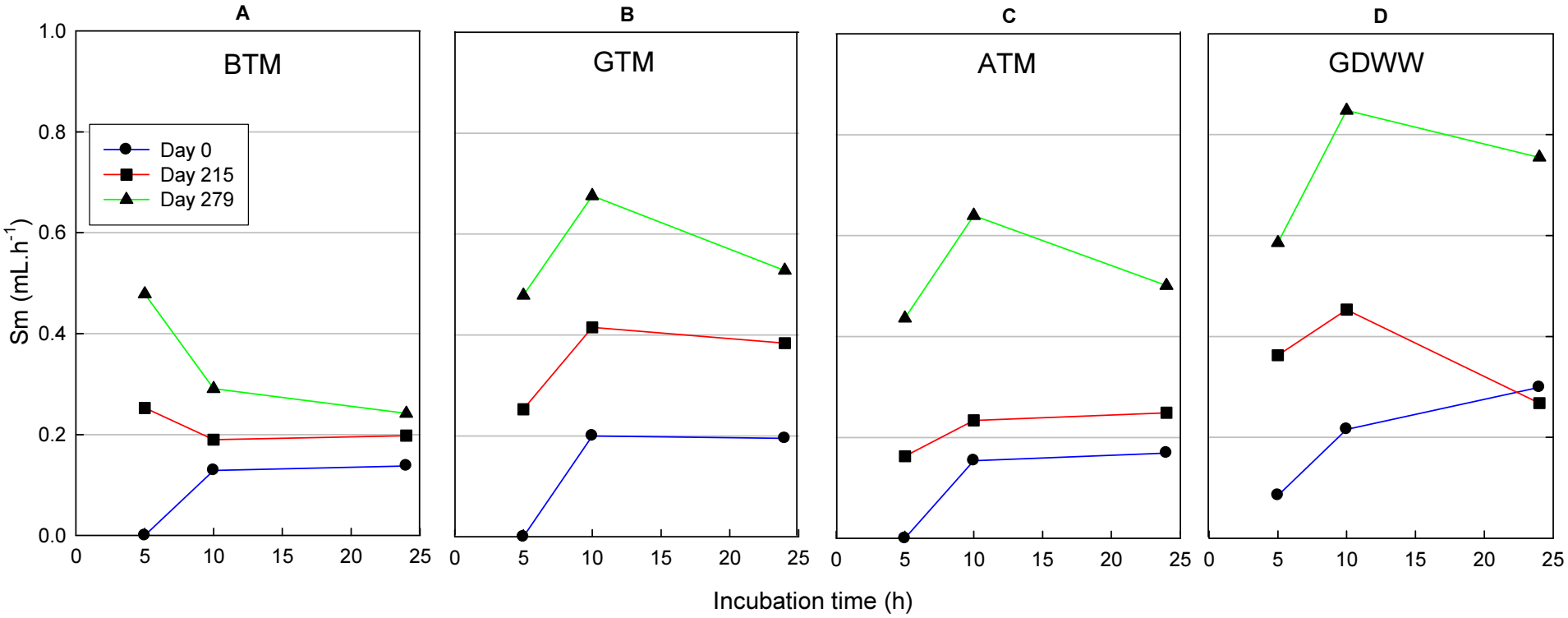


**Figure 5.7** Methane production rate ($mL.h^{-1}$) of the UASB granules on day 0, day 215 and day 279 after incubation in BTM, GTM, ATM and GDWW.

The cumulative biogas production increased to 5.98 mL (day 215) and 12.02 mL (day 279), respectively, indicating improved biogas production for acclimatised granules. Methane production rate (Fig. 5.7C) at 5 h shows increased activity in granules exposed to FOG-reduced GDWW for day 215 and 279 compared to day 0. The methane production was also higher at 24h for the exposed granules compared to the control granules. Additional acetic acid (1 $g.L^{-1}$) resulted in more substrate to convert and thus resulting in an improved methane production rate over 5 and 10 h incubation.

*FOG-reduced GDWW*

The FOG-reduced GDWW used as test medium could be a verification of the UASB granule's ability to acclimatise to this type of wastewater. The UASB granules exposed to FOG-reduced GDWW following the strategic dosing phase and from the continuous feeding phase (day 215 and 279, respectively) showed higher biogas production compared to initial (day 0) granules (Fig. 5.6D). Cumulative methane production after 24 h was initially 3.03 mL (day 0) (Fig. 5.6D). The cumulative methane production improved to 5.65 mL (day 215) and 11.1 mL (day 279) respectively, exhibiting improved methane production for granules exposed to FOG-reduced GDWW. The methane production rate (Fig. 5.7D) at 5 h also shows increased activity in granules exposed to FOG-reduced GDWW by day 215 and 279. The methane production rate was also higher at 24h for exposed granules (day 279).

The increased methane production is indicative of GDWW's ability of being a potential source of energy. The decrease in methane production rate in most of the test media after 10 h, especially at day 215 and 279, is a result of the faster production rate and the subsequent depletion of substrate taking place with less methane formation taking place as a result. The low initial cumulative methane production and methane production rate may be due to a lag phase experienced related to LCFA inhibition (Hwu *et al.*, 1998). The longer the unacclimatised granules are exposed to the medium more degradation (increased methane production) occurs. However, following the strategic feeding approach increased the efficiency of granules and the pace of GDWW degradation increased.

## CONCLUSION

The UASB reactor was used to treat FOG-reduced GDWW over a period of 279 days (two phases). The 1$^{st}$ phase of the study followed a novel technique to acclimatise the UASB reactor to the FOG-reduced GDWW by following a strategic feeding approach. The

reactor reached a COD load of 5 000 mg.$L^{-1}$ on day 216 (Phase A) and was continuously fed at this load (Phase B) until the end of the trial.

The strategic feeding approach resulted in the biomass successfully acclimatising to FOG-reduced GDWW. Granule activity tests (for BTM, GTM, ATM and GDWW) proved biomass acclimatisation of the strategically fed granules over seed granules in terms of methane production rate and cumulative methane production. Successful acclimatisation of the biomass to FOG-reduced GDWW resulted in improved UASB reactor stability in terms of COD reduction, FOG reduction, effluent pH and improved methane production when compared to the UASB reactor in Chapter 4. An additional granule activity test was performed on the UASB reactor biomass by the end of the investigation. The continuously fed granules showed the highest activity in terms of methane production rate and cumulative methane production over seed granules and granules after the strategic feeding. The increase in biomass activity as exposure time increased suggests a microbial population shift within the granule to efficiently degrade GDWW. This shows promise for further research into the possibility of further acclimatising the UASB reactor to GDWW and increasing the methane yield. This could be valuable to the optimisation of UASB reactors treating wastewaters containing FOG, thereby preventing reactor efficiency problems and possibly increasing the yield of renewable energy in the form of methane.

## REFERENCES

Alves, M., Vieira, J., Pereira, R., Pereira, M. & Mota, M. (2001). Effects of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors. Part II: Oleic acid toxicity and biodegradability. *Water Research*, **35**, 264 - 270.

APHA (1998). *Standard Methods for the Examination of Water and Wastewater*, 20th ed. Pp. 2:26-28, 22:54-58, 24:86-87, 24:149-150, 145:113-117, 145:134-139. Washington D.C.

Cammarota, M.C. & Freire, D.M.G. (2006). A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresource Technology*, **97**, 2195-2210.

Cavaleiro, A., Alves, J. & Alves, M. (2007). Discontinuous operation promotes efficient continuous anaerobic treatment of effluents with high lipid content. In: 11th IWA World Congress on Anaerobic Digestion. Brisbane, Australia.

Cavaleiro, A.J., Alves, M.M. & Mota, M. (2001). Microbial and operational response of an anaerobic fixed bed digester to oleic acid overloads. *Process Biochemistry*, **37**, 387-394.

Chernicharo, C.d.L. (2007). *Anaerobic Reactors.* Pp. 1 - 184. IWA Publishing.

Chipasa, K.B. & Mdrzycka, K. (2008). Characterization of the fate of lipids in activated sludge. *Journal of Environmental Sciences*, **20**, 536-542.

Deepak, L.J. (1998). High rate anaerobic treatment of industrial wastewater in tropics. *International Journal of Science and Technology*, **3**, 1 - 7.

Forday, W. & Greenfield, P.F. (1983). Anaerobic digestion. *Effluent and Water Treatment Journal*, **23**, 405 - 409.

Gavrilescu, M. (2002). Engineering concerns and new developments in anaerobic waste-water treatment. *Dynamic Biochemistry, Process Biotechnology and Molecular Biology*, **3**, 346-362.

Gerardi, M.H. (2003). *The Microbiology of Anaerobic Digesters.* Pp. 1 - 192. Wiley.

Hwu, C.-S., Tseng, S.-K., Yuan, C.-Y., Kulik, Z. & Lettinga, G. (1998). Biosorption of long-chain fatty acids in UASB treatment process. *Water Research*, **32**, 1571-1579.

Koster, I. & Cramer, A. (1987). Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids. *Applied & Environmental Microbiology*, **53**, 403-409.

Mendes, A. & Castro, H. (2005). Effect on the enzymatic hydrolysis of lipids from dairy wastewater by replacing Gum Arabic emulsifier for sodium chloride. *Brazilian Archives of Biology and Technology*, **48**, 135-142.

Miranda, L., Henriques, J. & Monteggia, L. (2005). A full-scale UASB reactor for treatment of pig and cattle slaughterhouse wastewater with a high oil and grease content. *Brazilian Journal of Chemical Engineering*, **22**, 601-610.

Mohana, S., Acharya, B.K. & Madamwar, D. (2009). Distillery spent wash: Treatment technologies and potential applications. *Journal of Hazardous Materials*, **163**, 12-25.

O' Kennedy, O.D. (2000). Application of biogranules in the anaerobic treatment of distillery effluents. MSc in Food Science Thesis, University of Stellenbosch,

Rinzema, A., Boone, M., van Knippenberg, K. & Lettinga, G. (1994). Bactericidal effect of long chain fatty acids in anaerobic digestion. *Water Environment Research*, **66**, 40-49.

Satyawali, Y. & Balakrishnan, M. (2007). Removal of color from biomethanated distillery spentwash by treatment with activated carbons. *Bioresource Technology*, **98**, 2629-2635.

Sigge, G. & Britz, T. (2007). UASB treatment of a highly alkaline fruit-cannery lye-peeling wastewater. *Water SA*, **33**, 275-278.

Tiwari, M., Guha, S., Harendranath, C. & Tripathi, S. (2006). Influence of extrinsic factors on granulation in UASB reactor. *Applied Microbiology and Biotechnology*, **71**, 145-154.

# CHAPTER 6

# GENERAL DISCUSSION AND CONCLUSION

## BACKGROUND

Governments worldwide, including South Africa, are setting stricter requirements for pollution control and there has been an increasing demand for more effective and novel treatment technologies (Lu *et al.*, 1995; Akunna & Clark, 2000; Mohana *et al.*, 2009). Grain distillery wastewater shares the same characteristics of other distillery wastewaters, however, it is also rich in fats, oils and grease (FOG) (Goodwin & Stuart, 1994; Uzal *et al.*, 2003; Gao *et al.*, 2007). Disposal of these types of wastewaters, untreated or partially treated can be hazardous to the environment.

Literature has shown that UASB reactors could successfully treat GDWW if strict monitoring is applied. Gao (2007) managed to achieve up to 97.3% COD reduction at a organic loading rate (OLR) of 5 to 48 $kgCOD.m^{-3}.d^{-1}$ and Goodwin (1994) effectively treated GDWW at OLR of 15 $kgCOD.m^{-3}.d^{-1}$. Anaerobic treatment of GDWW does present operational difficulty with the formation of a lipid coating around the granules resulting in sludge bed washout and the toxic effect of long chain fatty acids (LCFA) on acetoclastic methanogens and acetogens (Koster & Cramer, 1987; Mendes & Castro, 2005; Miranda *et al.*, 2005; Cammarota & Freire, 2006; Chipasa & Mdrzycka, 2008). Other researchers are proposing combined treatments (Borja & Banks, 1995; Dinsdale *et al.*, 2000; Manjunath *et al.*, 2000; Jeganathan *et al.*, 2006). Successful coagulation/flocculation treatments have been recorded by several researchers on FOG containing wastewater (Al-Mutairi *et al.*, 2004; Sarkar *et al.*, 2006). This mechanism of FOG reduction can be regarded as an effective stand-alone treatment. Type of coagulant/flocculant, pH of the wastewater, mixing retention time and method of separation are parameters that require consideration when using such a technique to full extend.

The objective of this study was to enhance the efficiency of an UASB reactor treating FOG-reduced GDWW. This was done by firstly using a coagulation/flocculation-centrifugation step to obtain FOG-reduced GDWW. Secondly, to optimise the efficiency of a lab-scale UASB reactor treating the FOG-reduced GDWW at pre-determined operational parameters (increased OLR and lower influent pH). At the same time the level of biomass acclimatisation, in terms of granule activity, was also monitored. Thirdly, the stability of the

granules in the UASB was optimised by investigating the effect of a strategic feeding approach on the COD and FOG degradation in the lab-scale UASB reactor.

## COAGULATION/FLOCCULATION OF GRAIN DISTILLERY WASTEWATER

The composition of GDWW can be problematic to biological treatment systems, if used as the primary treatment, due to its high COD (10 000 – 60 000 $mg.L^{-1}$) and FOG (*ca.* 1 200 to 1 950 $mg.L^{-1}$). To obtain FOG-reduced GDWW a coagulation/flocculation-centrifugation treatment was utilised and several commercially available coagulant/flocculant products were evaluated in terms of FOG and solids removal. The coagulation/flocculation-centrifugation combination achieving the best FOG removal was chosen to produce FOG-reduced GDWW for subsequent UASB treatment investigations.

The GDWW (ranging from 1250 – 1950 $mg.L^{-1}$ FOG) was treated with the following solutions at pre-determined concentrations: $FeCl_3$ (250 $mg.L^{-1}$), Ferrifloc 1820 (100 $mg.L^{-1}$), Ultrafloc 3800 (110 $mg.L^{-1}$), Ultrafloc 5000 (100 $mg.L^{-1}$). The pre-determined concentration of coagulant/flocculant was added to 1.5 L of GDWW, mixed and centrifuged. Additional evaluations included a double centrifugation step, where a treatment started with a centrifugation followed by a dosing of either $FeCl_3$ or Ferrifloc 1820 and a subsequent centrifugation. A single centrifugation step, without the addition of any coagulant/flocculant was also evaluated.

The decanted GDWW after centrifugation had a reduced FOG concentration for all treatments evaluated. This is important for the operational problems that excessive FOG can cause during anaerobic digestion. A centrifugation followed by a $FeCl_3$ treatment resulted in the most consistent FOG and TSS removal efficiency, ranging from 91 to 98% and 82 to 94%, respectively. A single centrifugation and $FeCl_3$ had a FOG and TSS removal efficiency of 91 - 97% and 74 - 93%, respectively. A stand-alone centrifugation step showed the worst removal efficiency with a FOG and TSS removal efficiency ranging from 50 to 66% and 30 to 72%, respectively.

Based on the data obtained from this investigation it was decided to use $FeCl_3$ in combination with a single centrifugation step as pre-treatment (91 – 97% FOG and 74 – 93% TSS removal) for subsequent UASB investigations. Build-up of metal can occur in the sludge generated during pre-treatment and can be undesirable if sludge is used as animal feed, due to its toxic effects at higher concentrations (Xu *et al.*, 2001). Disposal or reuse thereof will require further investigation. This investigation only focused on the removal of FOG and solids from GDWW for subsequent UASB treatment and other optimisation

parameters for evaluating coagulants/flocculants were not considered. Furthermore, a better understanding of GDWW composition is required to identify more suitable coagulants/flocculants. By improving the FOG and solids removal efficiency it can greatly enhance the effectiveness of a secondary treatment.

## UPFLOW ANAEROBIC SLUDGE BLANKET REACTOR EFFICIENCY TREATING FOG-REDUCED GDWW

The aim of this investigation covered three objectives. The first objective was to achieve lab-scale reactor start-up treating FOG-reduced GDWW and maintain an organic loading rate similar to that of a full-scale UASB reactor from a local distillery. The second objective was to determine whether a lab-scale reactor's efficiency could be maintained or increased in terms of a higher organic loading rate and lowered substrate pH. Lastly, to determine the level of biomass acclimatisation of the lab-scale UASB reactor by performing a granule activity test and comparing it with initial granule activity.

The initial start-up objective was successful with the UASB reactor attaining an OLR of *ca.* 5.5 kgCOD.m$^{-3}$.d$^{-1}$ by day 60 treating FOG-reduced GDWW. Whilst maintaining the OLR in the range of *ca.* 5.5 kgCOD.m$^{-3}$.d$^{-1}$ and substrate pH at 7.00 the UASB reactor was able to effectively reduce COD (ranging from 75 to 85%) and FOG ( in the region of 60%). After the successful attainment of reactor start-up it was decided to investigate whether the UASB reactor could maintain or improve its efficiency in terms of an higher OLR and lower substrate pH. The OLR was progressively increased to 10 kgCOD.m$^{-3}$.d$^{-1}$ whilst substrate pH was lowered to 6.50. Combined lowering of substrate pH and too large increases in OLR resulted in UASB reactor efficiency deteriorating, as alkalinity levels started to fluctuate below normal operating conditions, increases in VFA and subsequent lower effluent pH were observed. This all resulted in COD reduction and FOG reduction decreasing to 43 and 50%, respectively. Sub-optimal reactor conditions resulted in an adjustment of the substrate pH to 7.50 having to be made, whilst still increasing the OLR. With the attainment of an OLR of 10 kgCOD.m$^{-3}$.d$^{-1}$ at an substrate pH of 7.50 the reactor was able to achieve COD and FOG reductions in the region of 80 and 60%, respectively. With the OLR in the region of *ca.* 10 kgCOD.m$^{-3}$.d$^{-1}$ the substrate pH was decreased progressively to 6.50. The UASB reactor was able to successfully operate at these conditions with COD and FOG reduction of 90 and 60%, respectively. The FOG reduction efficiency did not improve to such an extent as expected during UASB reactor feeding. This suggests the complexity of breaking down FOG in GDWW have rate limiting steps

and further investigation is required to improve the efficiency. Lastly, a granule activity test was performed and granules from the UASB reactor treating FOG-reduced GDWW were compared to initial seed granules. The granules showed increased activity over seed granules in terms of methane production rate and cumulative methane production. The FOG-reduced GDWW test media was indicative to what degree the microbial consortia from the granules have specialised to breakdown such a complex wastewater.

## EFFECT OF A FEEDING STRATEGY ON THE UASB REACTOR EFFICIENCY TREATING FOG-REDUCED GDWW

The aim of this investigation was to step-wise increase the COD and FOG degradation capabilities of a lab-scale UASB reactor treating FOG-reduced GDWW. The UASB reactor was started up with FOG-reduced GDWW by following a strategic feeding approach, including cycles of feeding followed by cycles of starvation. Each feeding cycle attained a higher COD load until the desired COD load was attained. After the desired COD loading was attained the UASB reactor was fed continuously and operational stability of the UASB reactor evaluated.

The UASB reactor reached an OLR of 5 $kgCOD.m^{-3}.d^{-1}$ after a period of 216 days following the unique feeding strategy. Throughout the feeding/starvation cycles the UASB reactor was able to maintain COD reduction in the region of 80% whereas FOG reduction capability improved, attaining 66% by the end of the feeding strategy. The strategic feeding approach resulted in the biomass successfully acclimatising to FOG-reduced GDWW. Granule activity tests (for BTM, GTM, ATM and FOG-reduced GDWW) proved biomass acclimatisation of the strategically fed granules over seed granules in terms of methane production rate and cumulative methane production. Successful acclimatisation of the biomass to FOG-reduced GDWW resulted in improved UASB reactor stability in terms of COD reduction, FOG reduction, effluent pH and improved methane production when compared to the UASB reactor in the previous investigation. An additional granule activity test was performed on the UASB reactor biomass by the end of the investigation. The continuously fed granules showed the highest activity in terms of methane production rate and cumulative methane production over seed granules and granules after the strategic feeding. The increase in biomass activity as exposure time increased suggests a microbial population shift within the granule to efficiently degrade GDWW. This shows promise for further research into the possibility of further acclimatising the UASB reactor to GDWW and increasing the methane yield. This could be valuable to the optimisation of

UASB reactors treating wastewaters containing FOG, thereby preventing reactor efficiency problems and possibly increasing the yield of renewable energy in the form of methane.

## CONCLUDING REMARKS AND FUTURE RESEARCH

Results have shown that a coagulation/flocculation-centrifugation step is an option to consider as a pre-treatment when treating GDWW in order to remove sufficient amounts of FOG and solids. It was concluded that a combined treatment of coagulation/flocculation-centrifugation followed by a UASB reactor treatment could be successfully maintained. Parameters such as OLR, pH (substrate and effluent), VFA, Alkalinity and FOG levels (substrate and effluent) must be closely monitored to avoid any interruptions from occurring. Following a strategic feeding strategy also proved successful, inducing biomass acclimatisation and more stable UASB reactor efficiency treating FOG-reduced GDWW.

Better understanding of the wastewater characteristics is required to develop a more effective coagulation/flocculation product and subsequently improve FOG removal efficiency. Mixing methodology also needs development to successfully adapt this treatment in a large-scale operation. This type of system will need to achieve minimal mixing time and maximise the FOG removal efficiency. It will also have to be a semi-continuous system to prevent becoming a bottleneck in a large scale operation.

It is important for any biologically based treatment system to successfully acclimatise to any type of wastewater and achieve efficient removal efficiency. This will result in time savings of start-up and improve removal efficiency. Further investigation is required whether a fully acclimatised system can utilise removed FOG from pre-treatment without a decrease in efficiency. This will have cost saving implications regarding treatment costs and the additional substrate for the biomass will increase biogas production. It must also be investigated whether bioaugmentation (development of a specialised consortia of microorganisms specifically for the treatment of GDWW) can shorten the start-up time of UASB reactors treating GDWW as well as improve overall efficiency. FOG-rich GDWW has a high methane yield and this energy can be harvested and used as a renewable energy source for the generation of electricity.

## REFERENCES

Akunna, J.C. & Clark, M. (2000). Performance of a granular-bed anaerobic baffled reactor (GRABBR) treating whisky distillery wastewater. *Bioresource Technology*, **74**, 257-261.

Al-Mutairi, N.Z., Hamoda, M.F. & Al-Ghusain, I. (2004). Coagulant selection and sludge conditioning in a slaughterhouse wastewater treatment plant. *Bioresource Technology*, **95**, 115-119.

Borja, R. & Banks, C.J. (1995). Comparison of an Anaerobic Filter and an Anaerobic Fluidized Bed Reactor Treating Palm Oil Mill Effluent. *Process Biochemistry*, **30**, 511-521.

Cammarota, M.C. & Freire, D.M.G. (2006). A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresource Technology*, **97**, 2195-2210.

Chipasa, K.B. & Mdrzycka, K. (2008). Characterization of the fate of lipids in activated sludge. *Journal of Environmental Sciences*, **20**, 536-542.

Dinsdale, R.M., Hawkes, F.R. & Hawkes, D.L. (2000). Anaerobic digestion of short chain organic acids in an expanded granular sludge bed reactor. *Water Research*, **34**, 2433-2438.

Gao, M., She, Z. & Jin, C. (2007). Performance evaluation of a mesophilic (37 °C) upflow anaerobic sludge blanket reactor in treating distiller's grains wastewater. *Journal of Hazardous Materials*, **141**, 808-813.

Goodwin, J.S. & Stuart, J.B. (1994). Anaerobic digestion of malt whisky distillery pot ale using upflow anaerobic sludge blanket reactors. *Bioresource Technology*, **49**, 75-81.

Jeganathan, J., Nakhla, G. & Bassi, A. (2006). Novel hybrid PBR-UASB system for the treatment of high strength pet food wastewater. *Proceedings of the Water Environment Federation*, **2006**, 5040-5048.

Koster, I. & Cramer, A. (1987). Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids. *Applied and environmental microbiology*, **53**, 403-409.

Lu, C., Yeh, A.C. & Lin, M.-R. (1995). Treatment of high-strength organic wastewaters using an anaerobic rotating biological contactor. *Environment International*, **21**, 313-323.

Manjunath, N.T., Mehrotra, I. & Mathur, R.P. (2000). Treatment of wastewater from slaughterhouse by DAF-UASB system. *Water Research*, **34**, 1930-1936.

Mendes, A. & Castro, H. (2005). Effect on the enzymatic hydrolysis of lipids from dairy wastewater by replacing Gum Arabic emulsifier for sodium chloride. *Brazilian Archives of Biology and Technology*, **48**, 135-142.

Miranda, L., Henriques, J. & Monteggia, L. (2005). A full-scale UASB reactor for treatment of pig and cattle slaughterhouse wastewater with a high oil and grease content. *Brazilian Journal of Chemical Engineering*, **22**, 601-610.

Mohana, S., Acharya, B.K. & Madamwar, D. (2009). Distillery spent wash: Treatment technologies and potential applications. *Journal of Hazardous Materials*, **163**, 12-25.

Sarkar, B., Chakrabarti, P., Vijaykumar, A. & Kale, V. (2006). Wastewater treatment in dairy industries—possibility of reuse. *Desalination*, **195**, 141-152.

Uzal, N., Gökçay, C.F. & Demirer, G.N. (2003). Sequential (anaerobic/aerobic) biological treatment of malt whisky wastewater. *Process Biochemistry*, **39**, 279-286.

Xu, L., Sheldon, B., Carawan, R., Larick, D. & Chao, A. (2001). Recovery and characterization of by-products from egg processing plant wastewater using coagulants. *Poultry Science*, **80**, 57.